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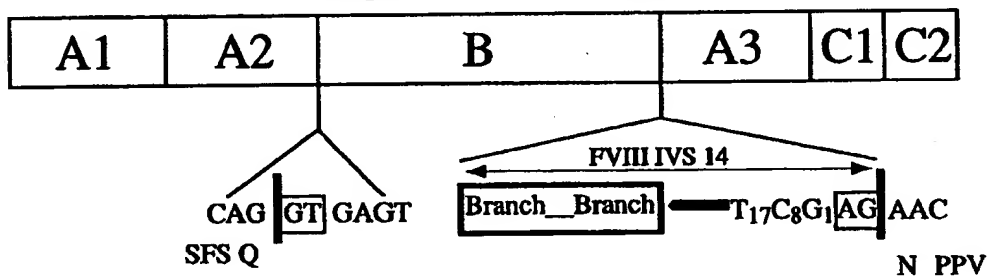
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(54) Title: NOVEL VECTORS AND GENES EXHIBITING INCREASED EXPRESSION

Full-length Factor VIII cDNA



(57) Abstract

Novel genes and vectors exhibiting increased expression and novel splicing patterns are disclosed. The gene can comprise one or more consensus or near consensus splice sites which have been corrected. The gene can alternatively or additionally comprise one or more introns within coding or noncoding sequences. The gene can still further comprise modified 5' and/or 3' untranslated regions optimized to provide high levels and duration of tissue-specific expression. In one embodiment, the gene comprises the coding region of a full-length Factor VIII gene modified by adding an intron within the portion of the gene encoding the  $\beta$ -domain, so that the gene is expressed as a  $\beta$ -domain deleted Factor VIII protein. The novel Factor VIII gene can also be modified to correct one or more consensus or near consensus splice sites within or outside of the coding region.

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## NOVEL VECTORS AND GENES EXHIBITING INCREASED EXPRESSION

### Background of the Invention

Recombinant DNA technology is currently the most valuable tool known for  
5 producing highly pure therapeutic proteins both *in vitro* and *in vivo* to treat clinical  
diseases. Accordingly, a vast number of genes encoding therapeutic proteins have been  
identified and cloned to date, providing valuable sources of protein. The value of these  
genes is, however, often limited by low expression levels.

This problem has traditionally been addressed using regulatory elements, such as  
10 optimal promoters and enhancers, which increase transcription/expression levels of  
genes. Additional techniques, particularly those which do not rely on foreign sequences  
(e.g., viral or other foreign regulatory elements) for increasing transcription efficiency of  
cloned genes, resulting in higher expression, would be of great value.

Accordingly, the present invention provides novel methods for increasing gene  
15 expression, and novel genes which exhibit such increased expression.

Gene expression begins with the process of transcription. Factors present in the  
cell nucleus bind to and transcribe DNA into RNA. This RNA (known as pre-mRNA) is  
then processed via splicing to remove non-coding regions, referred to as introns, prior to  
being exported out of the cell nucleus into the cytoplasm (where they are translated into  
20 protein). Thus, once spliced, pre-mRNA becomes mRNA which is free of introns and  
contains only coding sequences (i.e., exons) within its translated region.

Splicing of vertebrate pre-mRNAs occurs via a two step process involving splice  
site selection and subsequent excision of introns. Splice site selection is governed by  
definition of exons (Berget et al. (1995) *J. Biol. Chem.* 270(6):2411-2414), and begins  
25 with recognition by splicing factors, such as small nuclear ribonucleoproteins (snRNPs),  
of consensus sequences located at the 3' end of an intron (Green et al. (1986) *Annu. Rev.*  
*Genet.* 20:671-708). These sequences include a 3' splice acceptor site, and associated  
branch and pyrimidine sequences located closely upstream of 3' splice acceptor site  
(Langford et al. (1983) *Cell* 33:519-527). Once bound to the 3' splice acceptor site,  
30 splicing factors search downstream through the neighboring exon for a 5' splice donor  
site. For internal introns, if a 5' splice donor site is found within about 50 to 300  
nucleotides downstream of the 3' splice acceptor site, then the 5' splice donor site will  
generally be selected to define the exon (Robberson et al. (1990) *Mol. Cell. Biol.*  
10(1):84-94), beginning the process of spliceosome assembly.

35 Accordingly, splicing factors which bind to 3' splice acceptor and 5' splice donor  
sites communicate across exons to define these exons as the original units of  
spliceosome assembly, preceding excision of introns. Typically, stable exon complexes

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will only form and internal introns thereafter be defined if the exon is flanked by both a 3' splice acceptor site and 5' splice donor site, positioned in the correct orientation and within 50 to 300 nucleotides of one another.

It has also been shown that the searching mechanism defining exons is not a strict 5' to 3' (i.e., downstream) scan, but instead operates to find the "best fit" to consensus sequence (Robberson et al., *supra*. at page 92). For example, if a near-consensus 5' splice donor site is located between about 50 to 300 nucleotides downstream of a 3' splice acceptor site, it may still be selected to define an exon, even if it is not consensus. This may explain the variety of different splicing patterns (referred to as "alternative splicing") which is observed for many genes.

### Summary of the Invention

The present invention provides novel DNAs which exhibit increased expression of a protein of interest. The novel DNAs also can be characterized by increased levels of cytoplasmic mRNA accumulation following transcription within a cell, and by novel splicing patterns. The present invention also provides expression vectors which provide high tissue-specific expression of DNAs, and compositions for delivering such vectors to cells. The invention further provides methods of increasing gene expression and/or modifying the transcription pattern of a gene. The invention still further provides methods of producing a protein by recombinant expression of a novel DNA of the invention.

In one embodiment, a novel DNA of the invention comprises an isolated DNA (e.g., gene clone or cDNA) containing one or more consensus or near consensus splice sites (3' splice acceptor or 5' splice donor) which have been corrected. Such consensus or near consensus splice sites can be corrected by, for example, mutation (e.g., substitution) of at least one consensus nucleotide with a different, preferably non-consensus, nucleotide. These consensus nucleotides can be located within a consensus or near consensus splice site, or within an associated branch sequence (e.g., located upstream of a 3' splice acceptor site). Preferred consensus nucleotides for correction include invariant (i.e., conserved) nucleotides, including one or both of the invariant bases (AG) present in a 3' splice acceptor site; one or both of the invariant bases (GT) present in a 5' splice donor site; or the invariant A present in the branch sequence of a 3' splice acceptor site.

If the consensus or near consensus splice site is located within the coding region of a gene, then the correction is preferably achieved by conservative mutation. In a particularly preferred embodiment, all possible conservative mutations are made within a given consensus or near consensus splice site, so that the consensus or near consensus



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splice site is as far from consensus as possible (i.e., has the least homology to consensus as is possible) without changing the coding sequence of the consensus or near consensus splice site.

5 In another embodiment, a novel DNA of the invention comprises at least one non-naturally occurring intron, either within a coding sequence or within a 5' and/or 3' non-coding sequence of the DNA. Novel DNAs comprising one or more non-naturally occurring introns may further comprise one or more consensus or near consensus splice sites which have been corrected as previously summarized.

10 In a particular embodiment of the invention, the present invention provides a novel gene encoding a human Factor VIII protein. This novel gene comprises one or more non-naturally occurring introns which serve to increase transcription of the gene, or to alter splicing of the gene. The gene may alternatively or additionally comprise one or more consensus splice sites or near consensus splice sites which have been corrected, also to increase transcription of the gene, or to alter splicing of the gene. In one  
15 embodiment, the Factor VIII gene comprises the coding region of the full-length human Factor VIII gene, except that the coding region has been modified to contain an intron spanning, overlapping or within the region of the gene encoding the  $\beta$ -domain. This novel gene is therefore expressed as a  $\beta$ -domain deleted human Factor VIII protein, since all or a portion of the  $\beta$ -domain coding sequence (defined by an intron) is spliced  
20 out during transcription.

A particular novel human Factor VIII gene of the invention comprises the nucleotide sequence shown in SEQ ID NO:1. Another particular novel human Factor VIII gene of the invention comprises the coding region of the nucleotide sequence shown in SEQ ID NO:3 (nucleotides 1006-8237). Particular novel expression vectors  
25 of the invention comprise the complete nucleotide sequences shown in SEQ ID NOS: 2, 3 and 4. These vectors include novel 5' untranslated regulatory regions designed to provide high liver-specific expression of human Factor VIII protein.

In still other embodiments, the invention provides a method of increasing expression of a DNA sequence (e.g., a gene, such as a human Factor VIII gene), and a  
30 method of increasing the amount of mRNA which accumulates in the cytoplasm following transcription of a DNA sequence. In addition, the invention provides a method of altering the transcription pattern (e.g., splicing) of a DNA sequence. The methods of the present invention each involve correcting one or more consensus or near consensus splice sites within the nucleotide sequence of a DNA, and/or adding one or  
35 more non-naturally occurring introns into the nucleotide sequence of a DNA.

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In a particular embodiment, the invention provides a method of simultaneously increasing expression of a gene encoding human Factor VIII protein, while also altering the gene's splicing pattern. The method involves inserting into the coding region of the gene an intron which spans, overlaps or is contained within the portion of the gene encoding the  $\beta$ -domain. The method may additionally or alternatively comprise  
5 correcting within either the coding sequence or the 5' or 3' untranslated regions of the novel Factor VIII gene, one or more consensus or near consensus splice sites.

In yet another embodiment, the invention provides a method of producing a human Factor VIII protein, such as a  $\beta$ -domain deleted Factor VIII protein, by  
10 introducing an expression vector containing a novel human Factor VIII gene of the invention into a host cell capable of expressing the vector, under conditions appropriate for expression, and allowing for expression of the vector to occur.

#### **Brief Description of the Figures**

15 Figure 1 shows the nucleotide sequence of an RNA intron. The GU of the 5' splice donor site, the AG of the 3' splice acceptor site, and the A of the Branch are invariant bases (100% conserved and essential for recognition as splice sites). U is T in a DNA intron. The Branch sequence is located upstream from the 3' splice acceptor site at a distance sufficient to allow for lariat formation during spliceosome assembly  
20 (typically within 30-60 nucleotides). N is any nucleotide. Splicing will occur 5' of the GT base pair within the 5' splice donor site, and 3' of the AG base pair.

Figure 2 shows the conservative correction of a near consensus 3' splice acceptor site. The correction is made by silently mutating the A of the invariant (conserved) AG base pair to C, G, or T which does not affect the coding sequence of the intron because  
25 Ser is encoded by three alternate codons.

Figure 3 is a map of the coding region of a  $\beta$ -domain deleted human Factor VIII cDNA, showing the positions of the 99 silent point mutations which were made within the coding region (contained in plasmid pDJC) to conservatively correct all near consensus splice sites. Numbering of nucleotides begins with the ATG start coding of  
30 the coding sequence. Arrows above the map show positions mutated within near consensus 5' splice donor sites. Arrows below the map show positions mutated within near consensus 3' splice acceptor sites. Each "B" shown on the map shows a position mutated within a consensus branch sequence.

Figure 4A-4C shows the silent nucleotide substitution made at each of the 99  
35 positions made by arrows in Figure 3, as well as the codon containing the substitution and the amino acid encoded.

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Figure 5A-5O is a comparison of the coding sequence of (a) plasmid pDJC (top) containing the coding region of the human  $\beta$ -domain deleted Factor VIII cDNA modified by making 99 conservative point mutations to correct all near consensus splice sites within the coding region, and (b) plasmid p25D (bottom) containing the same coding sequence prior to making the 99 point mutations. Point mutations (substitutions) are indicated by a "v" between the two aligned sequences and correspond to the positions within the pDJC coding sequence shown in Figure 3. Plasmid p25D contains the same coding region as does plasmid pCY-2 shown in Figure 7 and referred to throughout the text.

Figure 6 shows a map of plasmid pDJC including restriction sites used for cloning, regulatory elements within the 5' untranslated region, and the corrected human  $\beta$ -domain deleted Factor VIII cDNA coding sequence.

Figure 7 shows a map of plasmid pCY-2 including restriction sites used for cloning, regulatory elements within the 5' untranslated region, and the uncorrected (i.e., naturally-occurring) human  $\beta$ -domain deleted Factor VIII cDNA coding sequence. pCY-2 and pDJC are identical except for their coding sequences.

Figure 8 is a map of the human  $\beta$ -domain deleted Factor VIII cDNA coding region showing the five sections of the cDNA (delineated by restriction sites) which can be synthesized (using overlapping 60-mer oligonucleotides) to contain corrected near consensus splice sites, and then assembled together to produce a new, corrected coding region.

Figure 9 is a schematic illustration of the cloning procedure used to insert an engineered intron into the coding region of the human Factor VIII cDNA, spanning a majority of the region of the cDNA encoding the  $\beta$ -domain. PCR fragments were generated containing nucleotide sequences necessary to create consensus 5' splice donor and 3' splice acceptor sites when cloned into selected positions flanking the  $\beta$ -domain coding sequence. The fragments were then cloned into plasmid pBluescript and sequenced. Once sequences had been confirmed, the fragments creating the 5' splice donor (SD) site were cloned into plasmid pCY-601 and pCY-6 (containing the full-length human Factor VIII cDNA coding region) immediately upstream of the  $\beta$ -domain coding sequence, and fragments creating the 3' splice acceptor (SA) site were cloned into pCY-601 and pCY-6 immediately downstream of the  $\beta$ -domain coding sequence. The resulting plasmids are referred to as pLZ-601 and pLZ-6, respectively.

Figure 10 is a map of the full-length human Factor VIII gene, showing the A1, A2, B, A3, C1 and C2 domains. Following expression of the gene, the  $\beta$  domain is naturally cleaved out of the protein. The map shows the 5' and 3' splice sites inserted within the B region of the gene (in plasmid pLZ-6) so that, during pre-mRNA

processing of the gene, the majority of the B region will be spliced out. Segments A2 and A3 of the gene will then be juxtaposed, coding for amino acids SFSQNPPV at the juncture.

Figure 11 shows the nucleotide sequences of the exon/intron boundaries (SEQ ID NO:5) flanking the  $\beta$ -domain coding region in plasmid pLZ-6 (containing the full-length human Factor VIII cDNA). The 5' splice donor site was added so that splicing would occur 5' of the "g" shown at position 2290. The 3' splice acceptor site was added so that splicing would occur 3' of the "g" shown at position 5147. Following splicing of the intron created by these splice sites, amino acids Gln-744 and Asn-1639 of the full-length human Factor VIII protein are brought together, resulting in a deletion of amino acids 745 to 1638 (numbering is in reference to Ala-1 of the mature human Factor VIII protein following cleavage of the 19 amino acid signal peptide). Capital letters represent nucleotide bases which remain within exons of the mRNA. Small case letters represent nucleotide bases which are spliced out of the mRNA as part of the intron.

Figure 12 is a map of the coding region of the full-length human Factor VIII gene showing (a) ATG (start) and TGA (stop) codons, (b) restriction sites within the coding region, (c) 5' splice donor (SD) and 3' splice acceptor (SA) sites of a rabbit  $\beta$ -globin intron positioned upstream of the coding region within the 5' untranslated region, (d) 5' splice donor and 3' splice acceptor sites added within the coding region defining an internal intron spanning the  $\beta$ -domain.

Figure 13 is a schematic illustration comparing the process of transcription, expression and post-translational modification for human Factor VIII produced from (a) a full-length human Factor VIII gene, (b) a  $\beta$ -domain deleted human Factor VIII gene, and (c) a full-length human Factor VIII gene containing an intron spanning the  $\beta$ -domain coding region.

Figure 14 is a graphic comparison of human Factor VIII expression for (a) pCY-6 (containing the coding region of the full-length human Factor VIII cDNA, as well as a 5' untranslated region derived from the second IVS of rabbit beta globin gene), (b) pCY-601 (containing the coding region of the full-length human Factor VIII cDNA, without the rabbit beta globin IVS), (c) pLZ-6 (containing the coding region of a full-length human Factor VIII cDNA with an intron spanning the  $\beta$ -domain, as well as the rabbit beta globin IVS), and (d) pLZ-601 (containing the coding region of a full-length human Factor VIII cDNA with an intron spanning the majority of the  $\beta$ -domain, without the rabbit beta globin IVS). Expression is given in nanograms. Transfection efficiencies were normalized to expression of human growth hormone (hGH). Each bar represents a summary of four separate transfection experiments.

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Figure 15 shows areas within the human Factor VIII transcription unit for sequence optimization.

Figure 16 shows the optimized intron-split leader sequence within vectors pCY-2, pCY-6, PLZ-6 and pCY2-SRE5, as well as the secondary structure of the leader sequence (SEQ ID NO:11) predicted by the computer program RNAdraw™.

Figure 17 is a schematic illustration showing two different RNA export pathways. The majority of mRNA's in higher eukaryotes contain intronic sequences which are removed within the nucleus (splicing pathway), followed by export of the mRNA into the cytoplasm. Mammalian intronless genes, hepadnaviruses (e.g., HBV), and many retroviruses access a nonsplicing pathway which is facilitated by cellular RNA export proteins (facilitated pathway).

Figure 18 is a graph showing the effect of a 5' intron and 3' post-transcriptional regulatory element (PRE) on human Factor VIII expression levels in HuH-7 cells. Plasmid pCY-2 contains a 5' intron but no PRE. Plasmid pCY-201 is identical to pCY-2, except that it lacks the 5' intron. Plasmid pCY-401 and pCY-402 are identical to pCY-201, except that they contain one and two copies of the PRE, respectively. The levels of secreted active Factor VIII was measured from supernatants collected 48 hours (first bar of each group) or 72 hours (second bar of each group) after transfection by Coatest VIII: c/4 kit from Kabi Inc. The transfection efficiency of each plasmid was normalized by analysis of human growth hormone secreted levels.

Figure 19 is a graph comparing human Factor VIII expression *in vivo* in mice for plasmids containing various regulatory elements upstream of either the  $\beta$ -domain deleted or full-length human Factor VIII gene. Plasmid pCY-2 has a 5' untranslated region containing the liver-specific thyroxin binding globulin (TBG) promoter, two copies of the liver-specific alpha-1 microglobulin/bikunin (ABP) enhancer; and a modified rabbit  $\beta$ -globin IVS, all upstream of the human  $\beta$ -domain deleted Factor VIII gene. Plasmid pCY2-SE5 is identical to pCY-2 except that the TBG promoter was replaced by the endothelium-specific human endothelin-1 (ET-1) gene promoter, and the ABP enhancers (both copies) were replaced by one copy of the human c-fos gene (SRE) enhancer. Plasmid pCY-6 is identical to pCY-2, except that the human  $\beta$ -domain deleted Factor VIII gene was replaced by the full-length human Factor VIII gene. Plasmid pLZ-6 is identical to pCY-6, except that the full-length human Factor VIII gene contained an intron spanning the  $\beta$ -domain. Plasmid pLZ-6A is identical to pLZ-6, except that it contains one corrected near consensus 3' splice acceptor site (A to C at base 3084 of pCY-6 (SEQ ID NO:3)). Each bar represents an average of five mice.

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Figure 20 shows the nucleotide sequence of the human alpha-1 microglobulin/bikunin (ABP) enhancer. Clustered liver-specific elements are underlined and labeled HNF-1, HNF-3 and HNF-4.

Figure 21 shows the nucleotide sequence of the human thyroxin binding globulin (TBG) promoter, also containing clustered liver-specific enhancer elements.

Figure 22 shows the nucleotide sequence and secondary structure of an optimized leader sequence.

Figure 23 is a comparison of the nucleotide sequences of the rabbit  $\beta$ -globin IVS before (top line) and after (bottom line) optimization to contain consensus 5' splice donor, 3' splice acceptor, branch, and translation initiation sites. Five nucleotides were also changed from purines to pyrimidines to optimize the pyrimidine track.

Figure 24 contains a list of various endothelium-specific promoters and enhancers, and characteristics associated with these promoters and enhancers.

Figure 25 is a graph comparing expression of plasmid pCY-2 and p25D *in vivo* in mice. Both plasmids contain the same coding sequence (for human  $\beta$ -domain deleted Factor VIII). Plasmid pCY-2 has an optimized 5' UTR containing two copies of the ABP enhancer, one copy of the TBG promoter and a leader sequence split by an optimized 5' rabbit  $\beta$ -globin intron. Plasmid p25D has a 5' UTR containing one copy of the CMV enhancer, one copy of the CMV promoter, and a leader sequence containing a short (130 bp) chimeric human IgE intron. Each bar represents an average of 5 mice.

## **Detailed Description of the Invention**

### **DEFINITIONS**

The present invention is described herein using the following terms which shall be understood to have the following meanings:

An "isolated DNA" means a DNA molecule removed from its natural sequence context (i.e., from its natural genome). The isolated DNA can be any DNA which is capable of being transcribed in a cell, including for example, a cloned gene (genomic or cDNA clone) encoding a protein of interest, operably linked to a promoter.

Alternatively, the isolated DNA can encode an antisense RNA.

A "5' consensus splice site" means a nucleotide sequence comprising the following bases: MAGGTRAGT, wherein M is (C or A), wherein R is (A or G) and wherein GT is essential for recognition as a 5' splice site (hereafter referred to as the "essential GT pair" or the "invariant GT pair").

A "3' consensus splice site" means a nucleotide sequence comprising the following bases (Y>8)NYAGG, wherein Y>8 is a pyrimidine track containing at least eight (most commonly twelve to fifteen or more) tandem pyrimidines (i.e., C or T (U if

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RNA)), wherein N comprises any nucleotide, wherein Y is a pyrimidine, and wherein the AG is essential for recognition as a 3' splice site (hereafter referred to as the "essential AG pair" or the "invariant AG pair"). A "3' consensus splice site" is also preceded upstream (at a sufficient distance to allow for lariat formation, typically at least about 40 bases) by a "branch sequence" comprising the following seven nucleotide bases: YNYTRAAY, wherein Y is a pyrimidine (C or T), N is any nucleotide, R is a purine (A or G), and A is essential for recognition as a branch sequence (hereafter referred to as "the essential A" or the "invariant A"). When all seven branch nucleotides are located consecutively in a row, the branch sequence is a "consensus branch sequence."

A "near consensus splice site" means a nucleotide sequence which:

- (a) comprises the essential 3' AT pair, and is at least about 50% homologous, more preferably at least about 60-70% homologous, and most preferably greater than 70% homologous to a 3' consensus splice site, when aligned with the consensus splice site for purposes of comparison; or
- (b) comprises the essential 5' GT pair, and is at least about 50% homologous, more preferably at least about 60-70% homologous, and most preferably greater than 70% homologous to a 5' consensus splice site, when aligned with the consensus splice site for purposes of comparison.

Homology refers to sequence similarity between two nucleic acids. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same nucleotide base, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences.

As will be described in more detail below, additional criteria for selecting "near consensus splice sites" can be used, adding to the definition provided above. For example, if a near consensus splice site shares homology with a 5' consensus splice site in only 5 out of 9 bases (i.e., about 55% homology), then these bases can be required to be located consecutively in a row. It can additionally or alternatively be required that a 3' near consensus splice site be preceded by a consensus branch sequence (i.e., no mismatches allowed), or followed downstream by a consensus or near consensus 5' splice donor site, to make the selection more stringent.

The term "corrected" as used herein refers to a near consensus splice site mutated by substitution of at least one nucleotide shared with a consensus splice site, hereafter referred to as a "consensus nucleotide". The consensus nucleotide within the near

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consensus splice site is substituted with a different, preferably non-consensus nucleotide. This makes the near consensus splice site "farther from consensus."

If the near consensus splice site is within a coding region of a gene, then the correction is preferably a conservative mutation. A "conservative mutation" means a  
5 base mutation which does not affect the amino acid sequence coded for, also known as a "silent mutation." Accordingly, in a preferred embodiment of the invention, correction of a near consensus splice site located within the coding region of a gene includes making all possible conservative mutations to consensus nucleotides within the site, so that the near consensus splice site is as far from consensus as possible without changing  
10 the amino acid sequence it encodes.

A "Factor VIII gene" as used herein means a gene (e.g., a cloned genomic gene or a cDNA) encoding a functional human Factor VIII protein from any species (e.g., human or mouse). A Factor VIII gene which is "full-length" comprises the complete coding sequence of the human Factor VIII gene found in nature, including the region  
15 encoding the  $\beta$ -domain. A Factor VIII gene which "encodes a  $\beta$ -domain deleted Factor VIII protein" or "a  $\beta$ -domain deleted Factor VIII gene" lacks all or a portion of the region of the full-length gene encoding the  $\beta$ -domain and, therefore, is transcribed and expressed as a "truncated" or " $\beta$ -domain deleted" Factor VIII protein. A gene which "is expressed as a  $\beta$ -domain deleted Factor VIII protein" includes not only a gene which  
20 encodes a  $\beta$ -domain deleted Factor VIII protein, but also a novel Factor VIII gene provided by the present invention which comprises the coding region of a full-length Factor VIII gene, except that it additionally contains an intron spanning the portion of the gene encoding the  $\beta$ -domain. The term "spans" means that the intron overlaps, encompasses, or is encompassed by the portion of the gene encoding the  $\beta$ -domain. The  
25 portion of the gene spanned by the intron is then spliced out of the gene during transcription, so that the resulting mRNA is expressed as a truncated or  $\beta$ -domain deleted Factor VIII protein.

A "truncated" or " $\beta$ -domain deleted" Factor VIII protein includes any active Factor VIII protein (human or otherwise) which contains a deletion of all or a portion of  
30 the  $\beta$ -domain..

A "non-naturally occurring intron" means an intron (defined by a 5' splice donor site and a 3' splice acceptor site) which has been engineered into a gene, and which is not present in the natural DNA or pre-mRNA nucleotide sequences of the gene.

An "expression vector" means any DNA vector (e.g., a plasmid vector)  
35 containing the necessary genetic elements for expression of a novel gene of the present invention. These elements, including a suitable promoter and preferably also a suitable enhancer, are "operably linked" to the gene, meaning that they are located at a position



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within the vector which enables them to have a functional effect on transcription of the gene.

#### **IDENTIFICATION OF CONSENSUS AND NEAR CONSENSUS SPLICE SITES**

5 A consensus or near consensus splice site can be identified within a DNA, or its corresponding RNA transcript, by evaluating the nucleotide sequence of the DNA for the presence of a sequence which is identical or highly homologous to either a 3' consensus splice acceptor site or a 5' consensus splice donor site (Figure 1). Such consensus and near consensus sites can be located within any portion of a given DNA  
10 (e.g., a gene), including the coding region of the DNA and any 3' and 5' untranslated regions.

To identify 3' consensus and near consensus splice acceptor sites, a DNA (or corresponding RNA) sequence is analyzed for the presence of one or more nucleotide sequences which includes an AG base pair, and which is either identical to or at least  
15 about 50% homologous, more preferably at least about 60-70% sequence homologous, to the sequence:  $(T/C) \geq 8 N(C/T) \underline{AGG}$ . In a preferred embodiment, the nucleotide sequence is also followed upstream, typically by about 40 bases, by a nucleotide sequence which is identical to or highly homologous (e.g., at least about 50%-95% homologous) to a branch consensus sequence comprising the following bases:  
20  $(C/T)N(C/T)T(A/G)\underline{A}(C/T)$ , wherein N is any nucleotide, and A is invariant (i.e., essential). By way of example, in studies described herein, consensus and near consensus 3' splice sites were selected for correction within a gene encoding Factor VIII using the following criteria: the consensus or near consensus site (a) contained an AG pair, and (b) contained no more than three mismatches to a 3' consensus site.

25 To identify 5' consensus and near consensus splice donor sites, a DNA (or corresponding RNA) sequence can be analyzed for the presence of one or more nucleotide sequences which contains a GT base pair, and which is either identical to or at least about 50% homologous, more preferably at least about 60-70% homologous, to the sequence:  $(A/C) \underline{AGGT} (A/G) AGT$ . By way of example, in studies described herein,  
30 consensus and near consensus 5' splice sites were selected for correction within a gene encoding Factor VIII using the following criteria: the consensus or near consensus site (a) contained a GT pair, and (b) contained no more than four mismatches to a 5' consensus site, provided that if it contained four mismatches, they were located consecutively in a row.

35 Evaluation of DNA or RNA sequences for the presence of one or more consensus or near consensus splice sites can be performed in any suitable manner. For example, nucleotide sequences can be manually analyzed. Alternatively, a computer

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algorithm can be employed to search nucleotide sequences for specified base patterns (e.g., the MacVector™ program). The latter approach is preferred for large DNAs or RNAs, particularly because it allows for easy implementation of multiple search parameters.

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### **CORRECTION OF CONSENSUS AND NEAR CONSENSUS SPLICE SITES**

In one embodiment of the invention, splice and branch sequences which are consensus, or near consensus, are corrected by substitution of one or more consensus nucleotides within the site. The consensus nucleotide within the site is preferably substituted with a non-consensus nucleotide. For example, if the nucleotide being substituted is a C (i.e., a pyrimidine) and the consensus sequence contains either C or T, then the nucleotide is preferably substituted by an A or G (i.e., a purine), thereby making the consensus or near consensus splice site "farther from consensus."

In a preferred embodiment of the invention, consensus and near consensus sites which are located within a coding region of a gene are corrected by conservative substitution of one or more nucleotides so that the correction does not affect the amino acid sequence coded for. Such conservative or "silent" mutation of codons to preserve coding sequences is well known in the art. Accordingly, the skilled artisan will be able to select appropriate base substitutions to retain the coding sequence of any codon which forms all or part of a consensus or near consensus splice site. For example, as shown in Figure 2, if a 3' near consensus splice site contains a TCA codon encoding serine, and the A is a consensus nucleotide (e.g., part of the essential AG pair, then this nucleotide can be substituted with a C, G, or a T to correct the 3' near consensus splice site (e.g., making it no longer near consensus because it does not contain the essential AG pair required for a 3' near consensus splice site), without affecting the coding sequence of the codon.

Accordingly, in a preferred embodiment of the invention, correction of consensus or near consensus splice sites which are specifically located within the coding region of a gene is achieved by substitution of one or both bases of an essential AG or GT pair within the consensus or near consensus splice site, with a base which does not alter the coding sequence of the site. Correction of consensus or near consensus branch sequences is similarly achieved by substitution of the essential A within the consensus or near consensus branch site, with a base which does not alter the coding sequence of the site. By correcting any of these essential bases, the splice or branch site will no longer be consensus or near consensus.

In another preferred embodiment, correction of consensus or near consensus splice sites which are specifically located within the coding region of a gene is achieved

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by making all possible conservative mutations to consensus nucleotides within the site, so that the consensus or near consensus splice site is as far from consensus as possible but encodes the same amino acid sequence.

Other preferred corrections of the invention include corrections of 3' consensus and near consensus splice sites which are followed downstream (e.g., by approximately 50-350 nucleotides) by a consensus or near consensus 5' splice donor site. Other preferred corrections of the invention include corrections of 5' consensus and near consensus splice sites which are preceded upstream (e.g., by about 50-350 nucleotides) by a consensus or near consensus 3' splice acceptor site.

For consensus or near consensus splice sites which are located outside the coding region of a gene, for example, in a 3' or 5' untranslated region (UTR), alternative approaches to correction can also be employed. For instance, because preservation of the coding sequence is not a consideration, the near consensus splice site can be corrected not only by any base substitution, but also by addition or deletion of one or more bases within the consensus or near consensus splice site, making the site farther from consensus.

Techniques for making nucleotide base substitutions, additions and deletions as described above are well known in the art. For example, standard point mutation may be employed to substitute one or more bases within a near consensus splice site with a different (e.g., non-consensus) base. Alternatively, as described in detail in the examples below, entire genes or portions thereof can be reconstructed (e.g., resynthesized using PCR), to correct multiple consensus and near consensus splice sites within a particular region of a gene. This approach is particularly advantageous if a gene contains a high concentration of consensus and/or near consensus splice sites within a given region.

In a specific embodiment, the invention features a novel Factor VIII gene containing one or more consensus or near consensus splice sites which have been corrected by substitution of one or more consensus nucleotides within the site. As part of the present invention, the coding region of a gene (cDNA) encoding human  $\beta$ -domain deleted Factor VIII protein (nucleotides 1006-5379 of SEQ ID NO:2) was evaluated as described herein and found to contain 23 near consensus 5' splice (donor) sequences, 22 near consensus 3' splice (acceptor) sequences, and 18 consensus branch sequences (shown in Figure 3). A new coding sequence (SEQ ID NO:1) was then developed for this gene to correct all 3' and 5' near consensus splice sites by conservative mutation. In total, 99 point mutations were made to the coding region. The location of each of these point mutations is shown in Figure 3. The specific base substitution made in each of these point mutations is shown in Figure 4(A-C). A comparison of this new coding

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sequence (SEQ ID NO:1) and the original uncorrected sequence (nucleotides 1006-5379 of SEQ ID NO:2), also showing the positions and specific substitutions made in each of the ninety-nine point mutations, is shown in Figure 5(A-O). A plasmid vector, referred to as pDJC, containing the new (i.e., corrected) Factor VIII gene coding sequence,  
5 including restriction sites used to synthesize the gene and regulatory elements used to express the gene, is shown in Figure 6. A plasmid vector, referred to as pCY2, containing the original, uncorrected Factor VIII gene, including restriction sites and regulatory elements used to express the gene, is shown in Figure 7.

As described in further detail in the examples below, all 99 consensus base  
10 corrections within the coding region of pDJC can be made by synthesizing overlapping oligonucleotides (based on the sequence of pCY2 shown in SEQ ID NO:2) which contain the desired corrections. A schematic illustration of this process is shown in Figures 8. In total, 185 overlapping 60-mer oligonucleotides can be synthesized, and assembled in five segments using the method of Stemmer et al. (1995) *Gene* 164: 49-53.  
15 Prior to assembly, each segment can be sequenced and tested in *in vitro* transfection assays (e.g., nuclear and cytoplasmic RNA analysis) in pCY2.

As an alternative to the "correct all" approach described above, selective correction of consensus and near consensus splice sites can also be employed. This involves selecting only (a) consensus sites, and near consensus splice sites which are  
20 close to consensus, and/or (b) consensus sites and near consensus sites which are located at positions which render these sites more likely to function as a splice donor or acceptor site. To select only nucleotide sequences which are complete consensus or which are close to consensus, evaluation of a given nucleotide sequence is limited to analyzing the nucleotide sequence for sequences which are identical to or are highly homologous (e.g.,  
25 greater than 70-80% homologous) to a 3' or 5' consensus splice site. To select only nucleotide sequences which are located at positions which render these sites more likely to function as a splice donor or acceptor site, the location of each 3' consensus or near consensus splice site must be evaluated with respect to the position of any neighboring  
30 5' consensus or near consensus splice sites. If a 3' consensus or near consensus splice site is located approximately 50-350 bases upstream from a 5' consensus or near consensus splice site, then these 3' and 5' splice sites are likely to function as a splice acceptor and donor sites. Therefore, these sites are preferably, and selectively, removed.

By way of example, particular consensus and/or near consensus 5' splice donor and 3' splice acceptor sites, as shown in Figure 3, can be selected within the coding  
35 region of the cDNA encoding human  $\beta$ -domain deleted Factor VIII (nucleotides 1006-5379 of SEQ ID NO:2) for preferred correction, based on their relative locations (i.e., 3' splice acceptor site located approximately 50-350 bases upstream from 5' near consensus

splice site). Such preferred selective corrections can include, for instance, the near consensus 3' splice acceptor site spanning nucleotide base 1851 of the coding region (see Figure 3) and any of the near consensus 5' splice donor sites located within 50-350 bases downstream of this near consensus 3' splice acceptor site, such as those spanning positions 1956, 1959, 2115, 2178 and 2184.

Splice site correction as provided herein can be applied to any gene known in the art. For example, the complete nucleotide sequence of other (e.g., full-length and  $\beta$ -domain deleted) Factor VIII genes (both genomic clones and cDNAs) are described in US Patent No. 4,757,006, US Patent No. 5,618,789, US Patent No. 5,683,905, and US Patent No. 4,868,112, the disclosures of which are incorporated by reference herein. The nucleotide sequences of these genes can be analyzed for consensus and near consensus splice sites, and thereafter corrected, using the guidelines and procedures provided herein.

In addition, other genes, particularly large genes containing several introns and exons, are also suitable candidates for splice site correction. Such genes, include, for example, the gene encoding Factor IX, or the cystic fibrosis transmembrane regulator (CFTR) gene described in US Patent No. 5,240,846, or nucleic acids encoding CFTR monomers, as described in US Patent No. 5,639,661. The disclosures of both of these patents are accordingly incorporated by reference herein.

#### ADDITION OF INTRONS

In another embodiment, a novel gene of the invention includes one or more non-naturally occurring introns which have been added to the gene to increase expression of the gene, or to alter the splicing pattern of the gene. The present invention provides the first known instance of gene engineering which involved adding a non-naturally-occurring intron within the coding sequence of a gene, particularly without affecting the activity of the protein encoded by the gene. The benefit of intron addition in this context is at least two-fold. First, as shown in Figure 14 in the context of the human Factor VIII gene, addition of one or more introns into a gene increases the expression of the gene compared to the same gene without the intron. Second, the intron, when placed within the coding sequence of the gene, can be used to beneficially alter the splicing pattern of the gene (e.g., so that a particular protein of interest is expressed), and/or to increase cytoplasmic accumulation of mRNA transcribed from the gene.

Novel genes of the present invention may also contain introns outside of the coding region of the gene. For example, introns may be added to the 3' or 5' non-coding regions of the gene (untranslated regions (UTRs)). In a preferred embodiment of the invention, an intron is added upstream of the gene in the 5' UTR, as shown in pDJC

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(Figure 6) and pCY2 (Figure 7). Such introns may include newly engineered introns or pre-existing introns. In a preferred embodiment of the invention, the intron is derived from the rabbit  $\beta$ -globin intron (IVS).

In a particular embodiment, the invention provides a novel human Factor VIII gene which includes within its coding region one or more introns. If the gene comprises the coding region of a full-length human Factor VIII gene, then at least one of these introns preferably spans (i.e., overlaps, encompasses or is encompassed by) the portion of the gene encoding the  $\beta$ -domain. This portion of the gene is then spliced out during transcription of the gene, so that the gene is expressed as a  $\beta$ -domain deleted protein (i.e., a Factor VIII protein lacking all or a portion of the  $\beta$ -domain).

A  $\beta$ -domain deleted human Factor VIII protein possesses known advantages over a full-length human Factor VIII protein (also known as human Factor VIII:C), including reduced immunogenicity (Toole et al. (1986) *PNAS* 83: 5939-5942). Moreover, it is well known that the  $\beta$ -domain is not needed for activity of the Factor VIII protein. Thus, a novel Factor VIII gene of the invention provides the dual benefit of (1) increased and (2) preferred protein expression.

Addition of one or more introns into a gene can be achieved by adding a 5' splice donor site and a 3' splice acceptor site (Figure 1) into the nucleotide sequence of the gene at a desired location. If the intron is being added to remove a portion of the coding sequence from the gene, then a 5' splice donor site is placed at the 5' end of the portion being removed (i.e., defined by the intron) and a 3' splice acceptor site is placed at the 3' end of the portion to be removed. Preferably, the 5' splice donor and 3' splice acceptor sequences are consensus, including the branch sequence located upstream of the 3' splice site, so that they will be favored (and more likely bound) by cellular splicing machinery over any surrounding near consensus splice sites.

As shown in Figure 1, splicing will occur 5' of the essential GT base pair within the 5' splice donor site, and 3' of the essential AG base pair within the 3' splice acceptor site. Thus, for introns added to coding sequences of genes, the intron is preferably designed to that, upon splicing, the coding sequence is unaffected. This can be done by designing and adding 5' splice donor and 3' splice acceptor sites which include only conservative (i.e., silent) changes to the nucleotide sequence of the gene, so that addition of these splice sites does not alter the coding sequence.

For example, as part of the present invention, an intron was engineered into the coding sequence of a full-length cDNA encoding human Factor VIII (1006-8061 of SEQ ID NO:4). The intron spanned the portion of the gene encoding the  $\beta$ -domain (nucleotides 2290-5147 of SEQ ID NO:4, encoding amino acid residues 745-1638). As described in the examples below, this intron was created by adding a 5' splice donor site

(100% consensus) so that splicing would occur immediately 5' of the coding sequence of the  $\beta$ -domain. A 3' splice acceptor site was also added so that splicing would occur immediately 3' of the coding sequence of the  $\beta$ -domain. Figure 11 shows the nucleotide sequences (SEQ ID NO:5) of the precise boundaries of the resulting intron that was added.

The nucleotide sequence for the 5' splice donor site of the added intron was derived from the pre-existing splice donor sequence found at the 5' end of IVS (Intron) 13 of genomic Factor VIII. This intron precedes exon 14, the exon which contains the sequence coding for the  $\beta$ -domain. The inserted sequence also contained the first nine bases of IVS 13 following the splice donor sequence.

The sequence for the 3' splice acceptor site was derived from the pre-existing splice acceptor sequence found at the 3' end of IVS 14 of genomic Factor VIII. This intron follows exon 14, the  $\beta$ -domain-containing exon. The inserted 3' splice acceptor site also contained 130 bases upstream of the splice acceptor in IVS 14. This upstream region contains at least two near-consensus branch sequences.

Thus, both the 3' and 5' engineered splice sites were designed to take advantage of pre-existing nucleotide sequences within the  $\beta$ -domain region of the human Factor VIII gene.

The 5' splice donor, 3' splice acceptor, and branch sequences of the added intron were further modified so that they were 100% consensus (i.e., congruent to their respective consensus splicing sequences). Modifications (e.g., base substitutions) were chosen so as to not alter the coding sequence of bases located upstream of the 5' splice site and downstream of the 3' splice site (i.e., flanking the boundaries of the intron). A map showing the various domains of the full-length Factor VIII gene, along with the 5' splice donor and 3' splice acceptor sites inserted into the gene, is shown in Figure 10. The complete nucleotide sequences of the intron boundaries (i.e., 5' splice donor and 3' splice acceptor) are shown in Figure 11 (SEQ ID NO:5). A map showing the location of the location of the 5' splice donor and 3' splice acceptor sites with respect to various restriction sites (used to clone in the sites) is shown in Figure 12. As shown schematically in Figure 13, the resulting novel Factor VIII gene, in contrast to a full-length Factor VIII gene or a gene encoding  $\beta$ -domain deleted Factor VIII, is transcribed as a pre-mRNA which contains the region encoding the  $\beta$ -domain, but is then spliced to remove the majority of this region, so that the resulting mRNA is expressed as a  $\beta$ -domain deleted protein. A complete expression plasmid (pLZ-6) containing the coding sequence of this novel Factor VIII gene, as well as an engineered 5' untranslated region containing regulatory elements designed to provide high, liver-specific expression,

comprises the nucleotide sequence shown in SEQ ID NO:3. Bases 1006-8237 of pLZ-6 (SEQ ID NO:3) correspond to the coding region of the novel Factor VIII gene.

Accordingly, in a preferred embodiment, the invention provides a novel Factor VIII gene comprising a non-naturally occurring intron spanning all or a portion of the  $\beta$ -domain region of the gene. In one embodiment, the gene comprises the coding region of the nucleotide sequence shown in SEQ ID NO:3. The gene may also contain further modifications, such as additional introns, or one or more corrected consensus or near consensus splice sites as described herein. In particular, the gene may further comprise one or more introns upstream of the coding sequence of the gene, within the 5' UTR. As shown in Figures 6 and 7, a preferred intron for insertion within this region is the rabbit  $\beta$ -globin intron (IVS). In addition, consensus and near consensus splice site corrections can be made to the gene, such as those shown in Figures 3 and 4(A-C).

#### **OPTIMIZATION OF 5' AND 3' UNTRANSLATED REGIONS FOR HIGH TISSUE-SPECIFIC GENE EXPRESSION**

Novel DNAs of the invention are preferably in a form suitable for transcription and/or expression by a cell. Generally, the DNA is contained in an appropriate vector (e.g., an expression vector), such as a plasmid, and is operably linked to appropriate genetic regulatory elements which are functional in the cell. Such regulatory sequences include, for example, enhancer and promoter sequences which drive transcription of the gene. The gene may also include appropriate signal and polyadenylation sequences which provide for trafficking of the encoded protein to intracellular destinations or export of the mRNA. The signal sequence may be a natural sequence of the protein or an exogenous sequence.

Suitable DNA vectors are known in the art and include, for example, DNA plasmids and transposable genetic elements containing the aforementioned genetic regulatory and processing sequences. Particular expression vectors which can be used in the invention include, but are not limited to, pUC vectors (e.g., pUC19) (University of California, San Francisco) pBR322, and pcDNA1 (InVitrogen, Inc.). An expression plasmid, pMT2LA8, encoding a  $\beta$ -domain deleted Factor VIII protein is described, for example, by Pitman et al. (1993) *Blood* 81(11):2925-2935). Entire coding sequences for these plasmid vectors are also provided herein (SEQ ID NOS: 4 and 2, respectively).

Suitable regulatory sequences required for gene transcription, translation, processing and secretion are art-recognized, and are selected to direct expression of the desired protein in an appropriate cell. Accordingly, the term "regulatory sequence", as used herein, includes any genetic element present 5' (upstream) or 3' (downstream) of the translated region of a gene and which control or affect expression of the gene, such



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as enhancer and promoter sequences (e.g., viral promoters, such as SV40 and CMV promoters). Such regulatory sequences are discussed, for example, in Goeddel, Gene expression Technology: Methods in Enzymology, page 185, Academic Press, San Diego, CA (1990), and can be selected by those of ordinary skill in the art for use in the present invention.

In a preferred embodiment of the invention, the 5' and/or 3' untranslated regions (UTRs) of a gene construct (e.g., a novel DNA of the invention) are optimized to provide high, tissue-specific expression. Such optimization can include, for example, selection of optimal tissue-specific promoters and enhancers, multimerization of genetic elements, insertion of one or more introns within or outside of the coding sequence, correction of near-consensus 5' splice donor and 3' splice acceptor sites within or outside of the coding sequence, optimization of transcription initiation and termination sites, insertion of RNA export elements, and addition of polyadenylation trimer cassettes to insulate transcription. In preferred embodiments of the invention, a combination of the aforementioned elements and sequence modifications are selected and engineered into the gene construct to provide optimized expression.

For many applications of human gene therapy, it is desirable to express proteins in the liver, which has the highest rate of protein synthesis per gram of tissue. For example, effective gene therapy for human Factor VIII requires sufficient levels and duration of protein expression in hepatocytes where Factor VIII is naturally produced, and/or in endothelial cells (ECs) where von Willebrand factor is produced, a protein which stabilizes the secretion of Factor VIII. Thus, in one embodiment, the invention provides a gene construct (e.g., expression vector) optimized to produce high levels and duration of liver-specific protein expression. In a particular embodiment, the invention provides a human Factor VIII gene construct, optimized to produce high levels and duration of liver-specific or endothelium-specific protein expression. This is achieved, for example, by selecting optimal liver-specific and endothelium-specific promoters and enhancers, and by combining these tissue-specific elements with other genetic elements and modifications to increase gene transcription.

Accordingly, for high levels and duration of gene expression in the liver, suitable promoters include, for example, promoters known to contain liver-specific elements. In one embodiment, the invention employs the thyroid binding globulin (TBG) promoter described by Hayashi et al. (1993) *Molec. Endocrinol.* 7:1049-1060. As shown in Figure 21, the TBG promoter contains hepatic nuclear factor (HNF) enhancer elements and provides the additional advantage of having a precisely mapped transcriptional start site. This allows insertion of a leader sequence, preferably optimized as described

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herein, between the promoter and the transcriptional start site. Figure 21 also shows the complete nucleotide sequence of the TBG promoter (SEQ ID NO:10).

For high levels and duration of gene expression in endothelium, suitable endothelium-specific promoters include, for example, the human endothelin-1 (ET-1) gene promoter described by Lee et al. (1990) *J. Biol. Chem.* 265(18), the fms-like tyrosine kinase promoter (Flt-1) described by Morishita et al. (1995) *J. Biol. Chem.* 270(46), the Tie-2 promoter described by Korhonen et al. (1995) *Blood* 86(5):1828-1835, and the nitric oxide synthase promoter described by Zhang et al. (1995) *J. Biol. Chem.* 270(25) (see Figure 24).

Promoters selected for use in the invention are preferably paired with a suitable ubiquitous or tissue-specific enhancer designed to augment transcription levels. For example, in one embodiment, a liver-specific promoter, such as the TBG promoter, is used in conjunction with a liver-specific enhancer. In a preferred embodiment, the invention employs one or more copies of the liver-specific alpha-1 microglobulin/bikunin (ABP) enhancer described by Rouet et al. (1992) *J. Biol. Chem.* 267:20765-20773, in combination with the TBG promoter. As shown in Figure 20, the ABP enhancer contains a cluster of HNF enhancer elements common to many liver-specific genes within a short nucleotide sequence, making it suitable to multimerize. When multimerized, the ABP enhancer generally exhibits increased activity and functions in either orientation within a gene construct.

Thus, in one embodiment, the invention provides an expression vector or DNA construct comprising one or more copies of a liver-specific or endothelium-specific promoter and a liver-specific or endothelium-specific enhancer, the promoter and enhancer being derived from different genes, such as thyroid binding globulin gene and the alpha-1 microglobulin/bikunin gene.

Alternatively, strong ubiquitous (i.e., non-tissue specific) enhancers can be used in conjunction with tissue-specific promoters, such as the TBG promoter or the ET-1 promoter, to achieve high levels and duration of tissue-specific expression. Such ubiquitous enhancers include, for example, the human c-fos (SRE) gene enhancer described by Treisman et al. (1986) *Cell* 46 which, when used in combination with liver-specific promoters (e.g., TBG) or endothelium-specific promoters (e.g., ET-1), provide high levels of tissue-specific expression, as demonstrated in studies described herein.

Accordingly, in a particular embodiment, the invention provides a gene construct which is optimized for specific expression in liver cells by inserting within its 5' untranslated region one or more copies of the ABP enhancer (preferably two copies) coupled upstream with the TBG promoter, as shown in Figure 15. Specific gene constructs, such as pCY2 and pDJC, containing these elements inserted upstream of the

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coding region for human Factor VIII ( $\beta$ -domain deleted and full-length with intron spanning the  $\beta$ -domain), are shown in Figures 6 and 7, respectively. In another particular embodiment, the gene construct is optimized for specific expression in endothelial cells by inserting within its 5' region one or more copies of the c-fos SRE enhancer, or an endothelial-specific enhancer (e.g., the human tissue factor (hTF/m) enhancer described by Parry et al. (1995) *Arterioscler. Thromb. Vasc. Biol.* 15:612-621) coupled upstream with the ET-1 promoter.

In addition to selecting optimal promoters and enhancers, optimization of a gene construct can include the use of other genetic elements within the transcriptional unit of the gene to increase and/or prolong expression. In one embodiment, one or more introns (e.g., non-naturally occurring introns) are inserted into the 5' or 3' untranslated region (UTR) of the gene. Introns from a broad variety of known genes (e.g., mammalian genes) can be used for this purpose. In one embodiment, the invention employs the first intron (IVS) from the rabbit  $\beta$ -globin gene comprising the nucleotide sequence shown in Figure 23 (SEQ ID NO:6).

In cases where the intron does not contain consensus 5' splice donor and 3' splice acceptor sites, or a consensus branch and pyrimidine track sequence, the intron is preferably optimized (modified) to render these sites completely consensus. This can be achieved, for example, by substituting one or more nucleotides within the 5' or 3' splice site, as previously described herein to render the site consensus. For example, when using the rabbit  $\beta$ -globin intron, the nucleotide sequence can be modified as shown in Figure 16 to render the 5' splice donor and 3' splice acceptor sites, and the pyrimidine track, entirely consensus. This can facilitate efficient transcription and export of the gene message out of the cell nucleus, thereby increasing expression. Exemplary nucleotide substitutions within the rabbit  $\beta$ -globin IVS which can be made to achieve this result are shown in Figure 23 which shows a comparison of the sequence for the unmodified (wild-type) rabbit  $\beta$ -globin intron (SEQ ID NO:6) and the same sequence modified to render the 5' splice donor and 3' splice acceptor sites, and the pyrimidine track, entirely consensus (SEQ ID NO:7).

When engineering one or more introns into the 5' UTR of a gene construct, the intron can be inserted into the leader sequence of the gene, as shown in Figures 15, 16 and 22. Accordingly, the intron can be inserted within the leader sequence, downstream from the promoter and enhancer elements. This can be done in conjunction with one or more additional modifications to the leader sequence, all of which serve to increase transcription, stability and export of mRNAs. Such additional modifications include, for example, optimizing the translation initiation site (Kozak et al. (1986) *Cell* 44:283)

and/or the secondary structure of the leader sequence (Kozak et al. (1994) *Molec. Biol.* 235:95).

Accordingly, in a preferred embodiment, the invention provides a gene construct which contains within its transcriptional unit, one or a combination of the foregoing genetic elements and sequence modifications designed to provide high levels and duration of gene expression, optionally in a tissue-specific manner. In a particular embodiment, the construct contains a gene encoding human Factor VIII (e.g.,  $\beta$ -domain deleted or full-length), having a 5' untranslated region which is optimized to provide significant levels and duration of liver-specific or endothelium-specific expression.

Particularly preferred gene constructs of the invention include, for example, those comprising the nucleotide sequences shown in SEQ ID NO:2 and SEQ ID NO:4, referred to herein respectively as pCY-2 and pLZ-6. These constructs contain the coding sequences for human  $\beta$ -domain deleted Factor VIII (pCY-2) and full-length human Factor VIII (containing an intron spanning the  $\beta$ -domain) (pLZ-6) downstream from an optimized 5' UTR designed to provide high levels and duration of human Factor VIII expression in liver cells. Other preferred gene constructs comprise the identical 5' UTR of pCY-2 and pLZ-6, in conjunction with coding sequences for other proteins desired to be expressed in the liver (e.g., other blood coagulation factors, such as human Factor IX).

As shown in Figures 7, 15 and 16, plasmids pCY-2 and pLZ-6 contain 5' UTRs comprising a novel combination of regulatory elements and sequence modifications shown herein to provide high levels and duration of human Factor VIII expression, both *in vitro* and *in vivo*, in liver cells. Specifically, each construct comprises within its 5' UTR sequentially from 5' to 3' (a) two copies of the ABP enhancer (SEQ ID NO:9), (b) one copy of the TBG promoter (SEQ ID NO:10), and (c) an optimized 71 nucleotide leader sequence (SEQ ID NO: 11) split by intron 1 of the rabbit  $\beta$ -globin gene. The intron is optimized to contain consensus splice acceptor, donor and pyrimidine track sites.

The leader sequence within the 5' UTR of pCY-2 and pLZ-6 also contains an optimized translation initiation site (SEQ ID NO: 8). Specifically, the human Factor VIII gene contains a cytosine at the +4 position, following the AUG start codon. This base was changed to a guanine, resulting in an amino acid change within the signal sequence of the protein from a glutamine to a glutamic acid. The leader sequence was further designed to have no RNA secondary structure, as predetermined by an RNA-folding algorithm (Figure 16) (Kozak et al. (1994) *J. Mol. Biol.* 235:95).

In addition to optimization of the 5' UTR of a gene construct, the 3' UTR can also be engineered to include one or more genetic elements or sequence modifications which

increase and/or prolong expression of the gene. For example, the 3' UTR can be modified to provide optimal RNA processing, export and mRNA stability. In one embodiment of the invention, this is done by increasing translational termination efficiency. In mammalian RNA's, translational termination is generally optimal if the  
5 base following the stop codon is a purine (McCaughan et al. (1995) *PNAS* 92:5431). In the case of the human Factor VIII gene, the UGA stop codon is followed by a guanine and is thus already optimal. However, in other gene constructs of the invention which do not naturally contain an optimized translational termination sequence, the termination sequence can be optimized using, for example, site directed mutagenesis, to substitute the  
10 base following the stop codon for a purine.

In particular gene constructs of the invention which contain the human Factor VIII gene, the 3' UTR can further be modified to remove one or more of the three pentamer sequences AUUUA present in the 3' UTR of the gene. This can increase the stability of the message. Alternatively, the 3' UTR of the human Factor VIII gene, or  
15 any gene having a short-lived messenger RNA, can be switched with the 3' UTR of a gene associated with a message having a longer lifespan.

Additional modifications for optimizing gene constructs of the invention include insertion of one or more poly A trimer cassettes for optimal polyadenylation and 3' end formation. These can be inserted within the 5' UTR or the 3' UTR of the gene. In a  
20 preferred embodiment, the gene construct is flanked on either side by a poly A trimer cassette, as shown in Figure 15. These cassettes can inhibit transcription originating outside of the desired promoter in the transcriptional unit, ensuring that transcription of the gene occurs only in the tissue where the promoter is active (Maxwell et al. (1989) *Biotechniques* 1989 3:276). Additionally, because the poly A trimer cassette functions  
25 in both orientations, i.e., on each DNA strand, it can be utilized at the 3' end of the gene for transcriptional termination and polyadenylation, as well as to inhibit bottom strand transcription and production of antisense RNA.

In further embodiments of the invention, gene optimization includes the addition of viral elements for accessing non-splicing RNA export pathways. The majority of  
30 mRNAs in higher eukaryotes contain intronic sequences which are removed within the nucleus, followed by export of the mRNA into the cytoplasm. This is referred to as the splicing pathway. However, as shown in Figure 17, mammalian intronless genes, hepadnaviruses (e.g., HBV), and many retroviruses access a nonsplicing pathway which is facilitated by cellular RNA export proteins and/or specific sequences within. This is  
35 referred to as the facilitated pathway.

In a particular embodiment, the gene construct is modified to include one or more copies of the post-transcriptional regulatory element (PRE) from hepatitis B virus.

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This 587 base pair element and its function to facilitate export of mRNAs from the nucleus, is described in U.S. Patent No. 5,744,326. Generally, the PRE element is placed within the 3' UTR of the gene, and can be inserted as two or more copies to further increase expression, as shown in Figure 18 (plasmid pCY-401 verses plasmid pCY-402).

Gene constructs (e.g., expression vectors) of the invention can still further include sequence elements which impart both an autonomous replication activity (i.e., so that when the cell replicates, the plasmid replicates as well) and nuclear retention as an episome. Generally, these sequence elements are included outside of the transcriptional unit of the gene construct. Suitable sequences include those functional in mammalian cells, such as the oriP sequence and EBNA-1 gene from the Epstein-Barr virus (Yates et al. (1985) *Nature* 313:812). Other suitable sequences include the *E. coli* origin of replication, as shown in Figures 6 and 7.

Gene constructs of the invention, such as pDJC, pCY-2, pCY-6, pLZ-6 and pCY2-SE5, have been described above, but are not intended to be limiting. Other novel constructs can be made in accordance with the guidelines provided herein, and are intended to be included within the scope of the present invention.

#### **INCREASED CYTOPLASMIC RNA ACCUMULATION AND EXPRESSION**

Novel DNAs (e.g., genes) of the present invention are modified to increase expression, for example, by facilitate cytoplasmic accumulation of mRNA transcribed from the DNA and by optimizing the 5' and 3' untranslated regions of the DNA. Accordingly, cytoplasmic mRNA accumulation and/or expression of the DNA is increased relative to the same DNA in unmodified form.

To evaluate (e.g., quantify) levels of nuclear or cytoplasmic mRNA accumulation obtained following transcription of novel DNAs and vectors of the invention, a variety of art recognized techniques can be employed, such as those described in Sambrook et al. "Molecular Cloning," 2d ed., and in the examples below. Such techniques include, for instance, Northern blot analysis, using total nuclear or cytoplasmic RNA. This assay can, optionally, be normalized using mRNA transcribed from a control gene, such as a gene encoding glyceraldehyde phosphate dehydrogenase (GAPDH). Levels of nuclear and cytoplasmic RNA accumulation can then be compared for novel DNAs of the invention to determine whether an increase has occurred following correction of one or more consensus or near consensus splice sites, and/or by addition of one or more non-naturally occurring introns into the DNA.

Novel DNAs of the invention can also be assayed for altered splicing patterns using similar techniques. For example, as described in the examples below, to

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determine whether a non-naturally occurring intron has been successfully incorporated into a DNA so that it is correctly spliced during mRNA processing, cytoplasmic mRNA can be assayed by Northern blot analysis, reverse transcriptase PCR (RT-PCR), or RNase protection assays. Such assays are used to determine the size of the mRNA produced from the novel DNA containing the non-naturally occurring intron. The size of the mRNA can then be compared to the size of the DNA with and without the intron to determine whether splicing has been achieved, and whether the splicing pattern corresponds to that expected based on the size of the added intron.

Alternatively, protein expressed from cytoplasmic RNA can be assayed by SDS-PAGE analysis and sequenced to confirm that correct splicing has been achieved.

To measure expression levels, novel DNAs of the invention can also be tested in a variety of art-recognized expression assays. Suitable expression assays, as illustrated in the examples provided below, include quantitative ELISA (Zatloukal et al. (1994) *PNAS* 91: 5148-5152), radioimmunoassay (RIA), and enzyme activity assays. When expression of Factor VIII protein is being measured, in particular, Factor VIII activity assays such as the KabiCoATest, (Kabi Inc., Sweden) can be employed to quantify expression.

#### **GENE DELIVERY TO CELLS**

Following insertion into an appropriate vector, novel DNAs of the invention can be delivered to cells either *in vitro* or *in vivo*. For example, the DNA can be transfected into cells *in vitro* using standard transfection techniques, such as calcium phosphate precipitation (O'Mahoney et al. (1994) *DNA & Cell Biol.* 13(12): 1227-1232). Alternatively, the gene can be delivered to cells *in vivo* by, for example, intravenous or intramuscular injection.

In one embodiment of the invention, the gene is targeted for delivery to a specific cell by linking the plasmid to a carrier molecule containing a ligand which binds to a component on the surface of a cell, thereby forming a polynucleotide-carrier complex. The carrier can further comprise a nucleic acid binding agent which noncovalently mediates linkage of the DNA to the ligand of the carrier molecule.

The carrier molecule of the polynucleotide-carrier complex performs at least two functions: (1) it binds the polynucleotide (e.g., the plasmid) in a manner which is sufficiently stable (either *in vivo*, *ex vivo*, or *in vitro*) to prevent significant uncoupling of the polynucleotide extracellularly prior to internalization by a target cell, and (2) it binds to a component on the surface of a target cell so that the polynucleotide-carrier complex is internalized by the cell. Generally, the carrier is made up of a cell-specific ligand and a cationic moiety which, for example are

conjugated. The cell-specific ligand binds to a cell surface component, such as a protein, polypeptide, carbohydrate, lipid or combination thereof. It typically binds to a cell surface receptor. The cationic moiety binds, e.g., electrostatically, to the polynucleotide.

- 5       The ligand of the carrier molecule can be any natural or synthetic ligand which binds a cell surface receptor. The ligand can be a protein, polypeptide, glycoprotein, glycopeptide, glycolipid or synthetic carbohydrate which has functional groups that are exposed sufficiently to be recognized by the cell surface component. It can also be a component of a biological organism such as a virus, cells (e.g.,  
10   mammalian, bacterial, protozoan).

- Alternatively, the ligand can comprise an antibody, antibody fragment (e.g., an F(ab')<sub>2</sub> fragment) or analogues thereof (e.g., single chain antibodies) which binds the cell surface component (see e.g., Chen et al. (1994) *FEBS Letters* 338:167-169, Ferkol et al. (1993) *J. Clin. Invest.* 92:2394-2400, and Rojanasakul et al. (1994)  
15   *Pharmaceutical Res.* 11(12):1731-1736). Such antibodies can be produced by standard procedures.

- Ligands useful in forming the carrier will vary according to the particular cell to be targeted. For targeting hepatocytes, proteins, polypeptides and synthetic compounds containing galactose-terminal carbohydrates, such as carbohydrate trees obtained from  
20   natural glycoproteins or chemically synthesized, can be used. For example, natural glycoproteins that either contain terminal galactose residues or can be enzymatically treated to expose terminal galactose residues (e.g., by chemical or enzymatic desialylation) can be used. In one embodiment, the ligand is an asialoglycoprotein, such as asialoorosomucoid, asialofetuin or desialylated vesicular stomatitis virus. In another  
25   embodiment, the ligand is a tri- or tetra-antennary carbohydrate moiety.

- Alternatively, suitable ligands for targeting hepatocytes can be prepared by chemically coupling galactose-terminal carbohydrates (e.g., galactose, mannose, lactose, arabinogalactan etc.) to nongalactose-bearing proteins or polypeptides (e.g., polycations) by, for example, reductive lactosamination. Methods of forming a broad variety of other  
30   synthetic glycoproteins having exposed terminal galactose residues, all of which can be used to target hepatocytes, are described, for example, by Chen et al. (1994) *Human Gene Therapy* 5:429-435 and Ferkol et al. (1993) *FASEB J.* 7: 1081-1091 (galactosylation of polycationic histones and albumins using EDC); Perales et al. (1994) *PNAS* 91:4086-4090 and Midoux et al. (1993) *Nucleic Acids Research* 21(4):871-878 (lactosylation and  
35   galactosylation of polylysine using  $\alpha$ -D-galactopyranosyl phenylisothiocyanate and 4-isothiocyanatophenyl  $\beta$ -D-lactoside); Martinez-Fong (1994) *Hepatology* 20(6):1602-1608 (lactosylation of polylysine using sodium cyanoborohydride and preparation of



asialofetuin-polylysine conjugates using SPDP); and Plank et al. (1992) *Bioconjugate Chem.* 3:533-539 (reductive coupling of four terminal galactose residues to a synthetic carrier peptide, followed by linking the carrier to polylysine using SPDP).

For targeting the polynucleotide-carrier complex to other cell surface  
5 receptors, the carrier component of the complex can comprise other types of ligands. For example, mannose can be used to target macrophages (lymphoma) and Kupffer cells, mannose 6-phosphate glycoproteins can be used to target fibroblasts (fibro-sarcoma), intrinsic factor-vitamin B12 and bile acids (See Kramer et al. (1992) *J. Biol. Chem.* 267:18598- 18604) can be used to target enterocytes, insulin can be  
10 used to target fat cells and muscle cells (see e.g., Rosenkranz et al. (1992) *Experimental Cell Research* 199:323-329 and Huckett et al. (1990) *Chemical Pharmacology* 40(2):253-263), transferrin can be used to target smooth muscle cells (see e.g., Wagner et al. (1990) *PNAS* 87:3410-3414 and U.S. Patent No. 5, 354,844 (Beug et al.)), Apolipoprotein E can be used to target nerve cells, and pulmonary  
15 surfactants, such as Protein A, can be used to target epithelial cells (see e.g., Ross et al. (1995) *Human Gene Therapy* 6:31-40).

The cationic moiety of the carrier molecule can be any positively charged species capable of electrostatically binding to negatively charged polynucleotides. Preferred cationic moieties for use in the carrier are polycations, such as polylysine (e.g., poly-L-  
20 lysine), polyarginine, polyornithine, spermine, basic proteins such as histones (Chen et al., *supra.*), avidin, protamines (see e.g., Wagner et al., *supra.*), modified albumin (i.e., N-acylurea albumin) (see e.g., Huckett et al., *supra.*) and polyamidoamine cascade polymers (see e.g., Haensler et al. (1993) *Bioconjugate Chem.* 4: 372-379). A preferred polycation is polylysine (e.g., ranging from 3,800 to 60,000 daltons). Other preferred  
25 cationic moieties for use in the carrier are cationic liposomes.

In one embodiment, the carrier comprises polylysine having a molecular weight of about 17,000 daltons (purchased as the hydrogen bromide salt having a MW of a 26,000 daltons), corresponding to a chain length of approximately 100-120 lysine residues. In another embodiment, the carrier comprises a polycation having a molecular  
30 weight of about 2,600 daltons (purchased as the hydrogen bromide salt having a MW of a 4,000 daltons), corresponding to a chain length of approximately 15-10 lysine residues.

The carrier can be formed by linking a cationic moiety and a cell-specific ligand using standard cross-linking reagents which are well known in the art. The linkage is  
35 typically covalent. A preferred linkage is a peptide bond. This can be formed with a water soluble carbodiimide, such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), as described by McKee et al (1994) *Bioconjugate Chem.* 5: 306-

311 or Jung, G. et al. (1981) Biochem. Biophys. Res. Commun. 101: 599-606 or Grabarek et al. (1990) Anal. Biochem. 185:131. Alternative linkages are disulfide bonds which can be formed using cross-linking reagents, such as N-Succinimidyl 3-(2-pyridyldithio)propionate (SPDP), N-hydroxysuccinimidyl ester of chlorambucil, N-Succinimidyl-(4-Iodoacetyl)aminobenzoate (SIAB), Sulfo-SIAB, and Sulfo-succinimidyl-4-maleimidophenyl-butyrate (Sulfo-SMPB). Strong noncovalent linkages, such as avidin-biotin interactions, can also be used to link cationic moieties to a variety of cell binding agents to form suitable carrier molecules.

The linkage reaction can be optimized for the particular cationic moiety and cell binding agent used to form the carrier. The optimal ratio (w:w) of cationic moiety to cell binding agent can be determined empirically. This ratio will vary with the size of the cationic moiety (e.g., polycation) being used in the carrier, and with the size of the polynucleotide to be complexed. However, this ratio generally ranges from about 0.2-5.0 (cationic moiety : ligand). Uncoupled components and aggregates can be separated from the carrier by molecular sieve or ion exchange chromatography (e.g., Aquapore™ cation exchange, Rainin).

In one embodiment of the invention, a carrier made up of a conjugate of asialoorosomucoid and polylysine is formed with the cross linking agent 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide. After dialysis, the conjugate can be separated from unconjugated components by preparative acid-urea polyacrylamide gel electrophoresis (pH 4-5).

Following formation of the carrier molecule, the polynucleotide (e.g., plasmid) is linked to the carrier so that (a) the polynucleotide is sufficiently stable (either *in vivo*, *ex vivo*, or *in vitro*) to prevent significant uncoupling of the polynucleotide extracellularly prior to internalization by the target cell, (b) the polynucleotide is released in functional form under appropriate conditions within the cell, (c) the polynucleotide is not damaged and (d) the carrier retains its capacity to bind to cells. Generally, the linkage between the carrier and the polynucleotide is noncovalent. Appropriate noncovalent bonds include, for example, electrostatic bonds, hydrogen bonds, hydrophobic bonds, anti-polynucleotide antibody binding, linkages mediated by intercalating agents, and streptavidin or avidin binding to polynucleotide-containing biotinylated nucleotides. However, the carrier can also be directly (e.g., covalently) linked to the polynucleotide using, for example, chemical cross-linking agents (e.g., as described in WO-A-91/04753 (Cetus Corp.), entitled "Conjugates of Antisense Oligonucleotides and Therapeutic Uses Thereof").

As described in Example 4, polynucleotide-carrier complexes can be formed by combining a solution containing carrier molecules with a solution containing a

polynucleotide to be complexed, preferably so that the resulting composition is isotonic (see Example 4).

### ADMINISTRATION

5 Novel DNAs of the invention can be administered to cells either *in vitro* or *in vivo* for transcription and/or expression therein.

For *in vitro* delivery, cultured cells can be incubated with the DNA in an appropriate medium under suitable transfection conditions, as is well known in the art.

10 For *in vivo* delivery (e.g., in methods of gene therapy) DNAs of the invention (preferably contained within a suitable expression vector) can be administered to a subject in a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier", as used herein, is intended to include any physiologically acceptable vehicle for stabilizing DNAs of the present invention for administration *in vivo*, including, for example, saline and aqueous buffer solutions, solvents, dispersion media, antibacterial  
15 and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media is incompatible with the polynucleotide-carrier complexes of the present invention, use thereof in a therapeutic composition is contemplated.

20 Accordingly, novel DNAs of the invention can be combined with pharmaceutically acceptable carriers to form a pharmaceutical composition. In all cases, the pharmaceutical composition must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as  
25 bacteria and fungi. Protection of the polynucleotide-carrier complexes from degradative enzymes (e.g., nucleases) can be achieved by including in the composition a protective coating or nuclease inhibitor. Prevention of the action of microorganisms can be achieved by various anti-bacterial and anti-fungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like.

30 Novel DNAs of the invention may be administered *in vivo* by any suitable route of administration. The appropriate dosage may vary according to the selected route of administration. The DNAs are preferably injected intravenously in solution containing a pharmaceutically acceptable carrier, as defined herein. Sterile injectable solutions can be prepared by incorporating the DNA in the required amount in an  
35 appropriate buffer with one or a combination of ingredients enumerated above or below, followed by filtered sterilization. Other suitable routes of administration

include intravascular, subcutaneous (including slow-release implants), topical and oral.

Appropriate dosages may be determined empirically, as is routinely practiced in the art. For example, mice can be administered dosages of up to 1.0 mg of DNA  
5 per 20 g of mouse, or about 1.0 mL of DNA in solution per 1.4 mL of mouse blood.

Administration of a novel DNA, or protein expressed therefrom, to a subject can be in any pharmacological form including a therapeutically active amount of DNA or protein, in combination with another therapeutic molecule. Administration of a therapeutically active amount of a pharmaceutical composition of the present  
10 invention is defined as an amount effective, at dosages and for periods of time necessary to achieve the desired result (e.g., an improvement in clinical symptoms). A therapeutically active amount of DNA or protein may vary according to factors such as the disease state, age, sex, and weight of the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, several  
15 divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

### USES

Novel DNAs of the present invention can be used to efficiently express a  
20 desired protein within a cell. Accordingly, such DNAs can be used in any context in which gene transcription and/or expression is desired.

In one embodiment, the DNA is used in a method of gene therapy to treat a clinical disorder. In another embodiment, the DNA is used in antisense therapy to produce sufficient levels of nuclear and/or cytoplasmic mRNA to inhibit expression  
25 of a gene. In another embodiment, the DNA is used to study RNA processing and/or gene regulation *in vitro* or *in vivo*. In another embodiment, the DNA is used to produce therapeutic or diagnostic proteins which can then be administered to patients as exogenous proteins.

Methods for increasing levels of cytoplasmic RNA accumulation and gene  
30 expression provided by the present invention can also be used for any and all of the foregoing purposes.

In a preferred embodiment, the invention provides a method of increasing expression of a gene encoding human Factor VIII. Accordingly, the invention also provides an improved method of human Factor VIII gene therapy involving  
35 administering to a patient afflicted with a disease characterized by a deficiency in Factor VIII a novel Factor VIII gene in an amount sufficient to treat the disease.

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In addition, the present invention provides a novel method for altering the transcription pattern of a DNA. By correcting one or more consensus or near consensus splice sites within the DNA, or by adding one or more introns to the DNA, the natural splicing pattern of the DNA will be modified and, at the same time, expression may be increased. Accordingly, methods of the invention can be used to tailor the transcription of a DNA so that a greater amount of a particular desired RNA species is transcribed and ultimately expressed, relative to other RNA species transcribed from the DNA (i.e., alternatively spliced RNAs).

Methods of the invention can also be used to modify the coding sequence of a given DNA, so that the structure of the protein expressed from the DNA is altered in a beneficial manner. For example, introns can be added to the DNA so that portions of the gene will be removed during transcription and, thus, not be expressed. Preferred gene portions for removal in this manner include those encoding, e.g., antigenic regions of a protein and/or regions not required for activity. Alternatively, or additionally, consensus or near consensus splice sites can be corrected within the DNA so that previously recognizable (i.e., operable) introns and exons are no longer recognized by a cells splicing machinery. This alters the coding sequence of the mRNA ultimately transcribed from the DNA, and can also facilitate its export from the nucleus to the cytoplasm where it can be expressed.

This invention is illustrated further by the following examples which should not be construed as further limiting the subject invention. The contents of all references and published patent applications cited throughout this application are hereby incorporated by reference.

25

## **EXAMPLES**

### **EXAMPLE 1 - Construction of a Human Factor VIII Gene Containing an Intron Spanning the $\beta$ -Domain**

A full-length human Factor VIII cDNA containing an intron spanning the section of the cDNA encoding amino acids 745-1638 (Figure 11) was constructed as described below. Amino acid numbering was designated starting with Met-1 of the mature human Factor VIII protein and, thus, does not include the 19 amino acid signal peptide of the protein. The  $\beta$ -domain region of a human Factor VIII protein is made up of 983 amino acids (Vehar et al. (1984) *Nature* 312: 337-342). Thus, the region of the cDNA spliced out during pre-mRNA processing corresponds to about 89% of the  $\beta$ -domain.

To select suitable sites for inserting the 5' splice donor (SD) and 3' splice acceptor (SA) sites, the sequence of the full-length Factor VIII cDNA expression

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plasmid pCY-6 (SEQ ID NO:4) was scanned for convenient restriction enzyme sites. Restriction sites were selected according to the following criteria: (a) they flanked and were in close proximity to the sites into which the splicing signals were to be introduced, so that any PCR fragment generated to fill in the region between these sites would have  
5 as little chance as possible for undesired point mutations introduced by the process of PCR; (b) they would cut the expression plasmid in as few places as possible, preferably only at the site flanking the region of splice site introduction.

The restriction sites chosen according to these criteria for cloning in the splice donor site were: Kpn I (base 2816 of the coding sequence of pCY-6, or base 3822 of the  
10 complete nucleotide sequence of pCY-6 provided in SEQ ID NO:4, since the first 1005 bases of this plasmid are non-coding bases), and Tth 1111 (base 3449 of the coding sequence of pCY-6, or base 4455 of the complete nucleotide sequence of pCY-6 shown in SEQ ID NO:4). The restriction sites chosen according to these criteria for cloning in the splice acceptor site were: Bcl I (bases 1407 and 5424 of the coding sequence of  
15 pCY-6, or bases 2413 and 6430 of the complete nucleotide sequence of pCY-6 shown in SEQ ID NO:4) and BspE 1 (base 7228 of the coding sequence of pCY-6, or base 8234 of the complete nucleotide sequence of pCY-6 shown in SEQ ID NO:4).

#### **Generation of Splice Donor Site**

20 A fragment containing the region of Factor VIII cDNA from the Kpn I site to the Tth 111 I site, with the above described splice donor sequence inserted at the appropriate spot, was then generated in the following manner:

A. PCR primers were designed, such that the top strand upstream primer (Fragment A top) would prime at the Kpn I site of full-length Factor VIII cDNA (Figure  
25 12), and the bottom strand downstream primer (Fragment A bottom) would prime at the site of insertion for the 5' splice donor. The bottom strand primer also contained the insertion sequence. These primers were used in a PCR reaction with pCIS-F8 (full-length Factor VIII cDNA expression plasmid) as template to yield "Fragment A," which contains the sequence spanning the region of Factor VIII cDNA from Kpn I to the splice  
30 donor insertion site, located at the 3' end of the fragment.

B. In similar fashion, "Fragment B" was generated using primer "Fragment B top," which contains the insertion sequence, and would prime at the insertion site of full-length Factor VIII cDNA, and primer "Fragment B bottom," which would prime at the Tth 111 I site of full-length Factor VIII cDNA. "Fragment B" contains the sequence  
35 spanning the region of Factor VIII cDNA from the splice donor insertion site to Tth111 I. The 5' splice donor insertion sequence was located at the 5' end of the fragment.

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C. Fragments A and B were run on a horizontal agarose gel, excised, and extracted, in order to purify them away from unincorporated nucleotides and primers.

D. These fragments were then combined in a PCR reaction using as primers "Fragment A top" and "Fragment B bottom." The regions at the 3' end of Fragment A and the 5' end of Fragment B overlapped because they were identical, and the final product of this reaction was a PCR fragment spanning the Factor VIII cDNA from Kpn I to Tth111 I, and containing the engineered splice donor at the insertion site, i.e., near the beginning of the coding region of the  $\beta$ -domain of Factor VIII. This fragment was designated "Fragment AB."

E. Fragment AB (an overlap PCR product) was cloned into the EcoR V site of pBluescript II SK(+) to yield clone pBS-SD (Figure 9), and the sequence of the insertion was then confirmed.

#### Generation of Splice Acceptor Site

A fragment containing the region of Factor VIII cDNA from the second Bcl I site to the BspE I site, with the above described splice acceptor sequence inserted at the appropriate spot, was generated in the following manner:

A. PCR primers were designed, such that the top strand upstream primer (Primer A) would prime at the second Bcl I site, and the bottom strand downstream primer (Primer B2) would prime at the insertion site for the 3' splice acceptor. The bottom strand primer also contained the restriction sites Mun I and BspE I. These primers were used in a PCR reaction with pCIS-F8 as template to yield "Fragment I," which contains the sequence spanning the region of Factor VIII cDNA from the Bcl I site to the insertion site, with the Mun I and BspE I sites located at the 3' end of the fragment.

B. In a similar fashion, "Fragment III" was generated using "Primer G3" which contains the restriction site BstE II, the splice acceptor recognition sequence (polypyrimidine tract followed by "CAG"), and primes at the insertion site for the splice acceptor; and "Primer H," which would prime the bottom strand at the BspE I site, so that the resulting fragment would contain the restriction site BstE II, the splice acceptor recognition site and sequence spanning the region of Factor VIII cDNA from the insertion site to BspE I.

C. "Fragment II," which contained the branch signals and IVS 14 sequence, was generated by designing four oligos (C2, D, E, and F3), two top and two bottom, which, when combined, would overlap each other by 21 to 22 bases, and when filled in and amplified under PCR conditions, would generate a fragment containing a Mun I site, 130 bases of the aforementioned IVS 14 sequence (including the 2 branch sequences at

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the 5' end of the 130 bases), and the cloning sites BstE II and BspE I. In addition, two small primers (CX and FX2) were designed that would prime at the very ends of the expected fragment, in order to increase amplification of full-length PCR product. All oligonucleotide primers were combined in a single PCR reaction, and the desired

5 fragment was generated.

D. All three fragments were cloned into the EcoR V site of pBluescript II SK(+), and their sequences were then confirmed.

E. Fragment II was isolated out of pBluescript as a Mun I to BspE I fragment, and cloned into the pBluescript-Fragment I clone at the corresponding sites, to  
10 yield clone pBS-FI/FII (Figure 9), Fragment III was isolated out of pBluescript as a BstE II to BspE I fragment, and cloned into the corresponding sites of pBS-FI/FII to yield pBS-FI/FII/FIII (Figure 9). This final bluescript clone contained the region spanning Factor VIII cDNA from the second Bcl I site to the BspE I site, and contained the IVS 14 and splice acceptor sequence inserted at the appropriate sites. The pBS-FI/FII/FIII  
15 clone was then sequenced.

#### **Cloning Splice Donor and Acceptor Sites into a Factor VIII cDNA Vector (pCY-6)**

Fragment AB and Fragment I/II/III were isolated out of pBluescript and cloned into pCY-6 in the following manner:

20 A. Fragment I/II/III was isolated from pBS-FI/FII/FIII as a Bcl I to BspE I fragment.

B. pCY-601 was digested to completion with BspE I, linearizing the plasmid. This linear DNA was partially digested with Bcl I for 5 minutes, and then immediately run on a gel. The band corresponding to a fragment which had been cut  
25 only at the BspE I and the second Bcl I site was isolated and extracted from the agarose gel. This isolated fragment was ligated to Fragment I/II/III and yielded pCY-601/FI/FII/FIII (Figure 9).

C. Fragment AB was isolated from pBS-SD as a Kpn I to Tth111 I fragment, and cloned into the corresponding sites of pCY-601/FI/FII/FIII to yield pLZ-601.

30 D. Plasmids pCY-6 and pLZ-601 were digested sequentially with enzymes Nco I and Sal I. The small fragment of the pCY-6 digest and the large fragment of the pLZ-601 digest were isolated and ligated together to yield plasmid pLZ-6, a second  $\beta$ -domain intron Factor VIII expression plasmid.

pCY-6 and pCY-601 are expression plasmids for full-length Factor VIII cDNA.  
35 The difference between the two is that the former contains an intron in the 5' untranslated region of the Factor VIII transcript, derived from the second IVS of rabbit beta globin gene. The latter lacks this engineered IVS. *In vitro* experiments have shown



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that pCY-601 yields undetectable levels of Factor VIII, while pCY-6 yields low but detectable Factor VIII levels.

#### **Expression Assays**

- 5 To test expression of the various Factor VIII cDNA plasmids including those created as described above, plasmids were transfected at a concentration of 2.0-2.5  $\mu$ g/ml into HuH-7 human carcinoma cells using the calcium phosphate precipitation method described by O'Mahoney et al. (1994) *DNA & Cell Biol.* 13(12): 1227-1232. Expression levels were measured using the KabiCoATest (Kabi Inc., Sweden). This is  
10 both a quantitative and a qualitative assay for measuring Factor VIII expression, because it measures enzymatic activity of Factor VIII.

#### **Reverse Transcriptase-PCR Analysis of Cells Transfected With Factor VIII**

##### **Expression Plasmids**

- 15 To confirm that the engineered intron spanning the  $\beta$ -domain of the Factor VIII cDNA in plasmid pLZ-6 resulted in proper splicing of the  $\beta$ -domain coding region, reverse transcriptase (RT)-PCR analysis was performed as follows:

HU7 cells in T-75 flasks were transfected via  $\text{CaPO}_4$  precipitation with 36  $\mu$ g of each of the following DNA plasmids:

- |    |       |   |
|----|-------|---|
| 20 | pCY-2 | $\beta$ -domain deleted human Factor VIII cDNA                            |
|    | pCY-6 | Full-length human Factor VIII cDNA  |
|    | pLZ-6 | Full length human Factor VIII cDNA with engineered $\beta$ -domain intron |

- 75 ng of pCMVhGH was co-transfected as a transfection control. Untransfected  
25 cells were grown alongside as a negative control.

Total RNA was isolated from cells 24 hours post-transfection using Gibco BRL Trizol reagent, according to the standard protocol included in product insert.

- RT-PCR Experiments were performed as follows: RT-PCR was performed on all RNA preps to characterize RNA. "Minus RT" PCR was performed on all RNA preps  
30 as a negative control (without RT, only DNA is amplified). PCR was performed on plasmids used in transfection assays to compare with RT-PCRs of the RNA preps. All RT-PCR was performed with Access RT-PCR system (Promega, Cat. #A1250). In each 50  $\mu$ l reaction, 1.0  $\mu$ g total RNA was used as template. Primer pairs were designed according to Factor VIII sequences as follows: the 5' primer anneals to the top strand of  
35 Factor VIII, about 250 base pairs upstream of the  $\beta$ -domain junction; while the 3' primer anneals to the bottom strand of Factor VIII, about 250 base pairs downstream of the  $\beta$ -domain junction.

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The nucleotide sequences of the primers used to characterize (i.e., confirm) the  $\beta$ -domain intron splicing were as follows:

5' primer TS 2921-2940: 5'TGG TCT ATG AAG ACA CAC TC<sup>3'</sup>  
(20 mer)

5 3' primer BS 6261-6280: 5'TGA GCC CTG TTT CTT AGA AC<sup>3'</sup>  
(20 mer)

RT-PCR files were set up according to manufacturer's recommendation:

48°C, 45 minutes; x1 cycle

94°C, 2 minutes; x1 cycle

10 94°C, 30 sec; x 40 cycles

60°C, 1 min; x 40 cycles

68°C, 2 min; x 40 cycles

68°C, 7 min; x 1 cycle

4°C, soak overnight

15 The data obtained from the RT-PCR assays demonstrated that engineered  $\beta$ -domain intron was spliced as predicted. The RT-PCR product (~500 bp) generated from pLZ-6 (containing the  $\beta$ -domain intron) was similar to that obtained from pCY-2 (containing  $\beta$ -domain deleted Factor VIII cDNA). The RT-PCR product observed for pCY-6 (containing the full length Factor VIII cDNA) yielded a much larger band (~3.3  
20 kb).

In the control groups, it was confirmed that DNA from the Huh-7 cells transfected with various Factor VIII constructs were consistent with regular PCR results of the corresponding plasmids. Background bands from untransfected Huh-7 cells were presumably contributed by cross-over during sample handling. This can be further  
25 investigated by using polyA<sup>+</sup> RNA as template, as well as by setting up RT-PCR with different primer sets.

#### **EXAMPLE 2 - Correction of Consensus and near Consensus Splice Sites Within a Human Factor VIII Gene**

30 Plasmid pCY-2, containing the coding region of the  $\beta$ -domain deleted human Factor VIII cDNA (nucleotides 1006-5379 of SEQ ID NO:2), was analyzed using the MacVector™ program for consensus and near consensus (a) splice donor sites, (b) splice acceptor sites and (c) branch sequences. Near consensus 5' splice donor sites were selected using the following criteria: sites were required to contain at least 5 out of the 9  
35 splice donor consensus bases (i.e., (C/A)AGGT(A/G)AGT), including the invariant GT, provided that if only 5 out of 9 bases were present, these 5 bases were located consecutively in a row. Near consensus 3' splice acceptor sites were selected using the

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following criteria: sites were required to contain at least 3 out of the following 14 splice acceptor consensus bases (Y=10)CAGG (wherein Y is a pyrimidine within the pyrimidine track), including the invariant AG. Only branch sequences which were 100% consensus were searched for.

5        Using these criteria, 23 near consensus 5' splice donor sequences, 22 near consensus 3' splice acceptor sequences, and 18 consensus branch sequences were identified. No consensus 5' splice donor or 3' splice acceptor sequences were identified. To correct these near consensus splice donor and acceptor sequences, and consensus branch sequences, it was first determined whether the invariant GT, AG, or A bases  
10        within the site could be substituted without changing the coding sequence of the site. If they could be, then these conservative (silent) substitutions were made, thereby rendering the site non-consensus (since the invariant bases are required for recognition as a splice site).

      If the invariant bases within selected consensus and near consensus sites could  
15        not be substituted without changing the coding sequence of the site (i.e., if no degeneracy existed for the amino acid sequence coded for), then the maximum number of silent point mutations were made to render the site as far from consensus as possible. All bases which contributed to homology of the consensus or near consensus site with the corresponding consensus sequence, and which were able to be conservatively  
20        substituted (with non-consensus bases), were mutated.

      Using these guidelines, 99 silent point mutations were selected, as shown in Figure 4A-4C. The positions of each of these silent point mutations is shown in Figure 3.

      To prepare a new pCY-2 human  $\beta$ -domain deleted Factor VIII cDNA coding  
25        sequence which contains the above-described corrections, the following procedure can be used:

      Overlapping 60-mer oligonucleotides can be synthesized based on the coding sequence of pCY2. Each of the 185 oligonucleotide contains the desired corrections. These oligonucleotides are then assembled in five segments (shown in Figure 9) using  
30        the method of Stemmer et al. (1995) *Gene* 164: 49-53. Prior to assembly, each segment can be sequenced and tested in *in vitro* transfection assays (nuclear and cytoplasmic RNA analysis) in pCY2. A schematic illustration of this process is shown in Figures 8. The plasmid containing the new corrected coding sequence is designated "pDJC."

      To test expression levels of pDJC, the plasmid can be transfected at a  
35        concentration of 2.0-2.5  $\mu$ g/ml into HuH-7 human carcinoma cells using any suitable transfection technique, such as the calcium phosphate precipitation method described by O'Mahoney et al. (1994) *DNA & Cell Biol.* 13(12): 1227-1232. Factor VIII expression

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can then be measured using the KabiCoATest (Kabi Inc., Sweden). This is both a quantitative and a qualitative assay for measuring Factor VIII expression, because it measures enzymatic activity of Factor VIII.

Alternatively, plasmids such as pDJC can be tested for *in vivo* expression using the procedure described below in Example 4.

### **EXAMPLE 3 - Optimized Expression Vectors**

Optimized expression vectors for liver-specific and endothelium-specific human Factor VIII expression were prepared and tested as follows:

10 The  $\beta$ -domain deleted human Factor VIII cDNA was obtained through Bayer Corporation in plasmid p25D, having a coding sequence corresponding to nucleotides 1006-5379 of SEQ ID NO:2. The human thyroid binding globulin promoter (TBG) (bases -382 to +3) was obtained by PCR from human liver genomic DNA (Hayashi et al. (1993) *Mol. Endo.* 7:1049). The human endothelin-1 (ET-1) gene promoter (Lee et al. 15 (1990) *J. Biol. Chem.* 265(18) was synthesized by amplification of overlapping oligos in a PCR reaction.

After sequence confirmation, the TBG and ET-1 promoters were cloned into two separate vectors upstream of an optimized leader sequence (SEQ ID NO:11), using standard cloning techniques. The leader sequence was designed in a similar manner to 20 that reported by Kozak et al. (1994) *J. Mol. Biol.* 235:95) and synthesized (Retrogen Inc., San Diego, CA) as 71 base pair top and bottom strand oligos, annealed and cloned upstream of the Factor VIII ATG. The 126 base pair intron-1 of the rabbit  $\beta$ -globin gene, containing the nucleotide sequence modifications shown in Figure 23 (SEQ ID NO:7), was also synthesized and inserted into the leader sequence following base 42 of the 71 25 nucleotide sequence.

In the construct containing the TBG promoter, top and bottom strands of the human alpha-1 microglobulin/bikunin enhancer (ABP), sequences -2804 through -2704 (Rouet et al. (1992) *J. Biol. Chem.* 267:20765), were synthesized, annealed and cloned upstream of the promoter. Cloning sites flanking the enhancer were designed to facilitate 30 easy multimerization. In the construct containing the ES-1 promoter, top and bottom strands of the human c-fos SRE enhancer (Treisman et al. (1986) *Cell* 46) were synthesized, annealed and cloned upstream of the promoter.

The post-transcriptional regulatory element (PRE) from hepatitis B virus, was isolated from plasmid Adw-HTD as a 587 base-pair Stu I-Stu I fragment. It was cloned 35 into the 3' UTR of the Factor VIII construct (at the Hpa I site) containing the TBG promoter and ABP enhancers, upstream of the polyadenylation sequence. A two copy PRE element was isolated as a Spe I-Spe I fragment from an early vector where two

copies had ligated together. This fragment was converted to a blunt end fragment by the Klenow fragment of E-coli DNA polymerase I and also cloned into the Factor VIII construct at the same Hpa I site.

Thus, the following constructs were produced using the foregoing materials and methods:

Plasmid pCY-2 having a 5' untranslated region containing the TBG promoter, two copies of the ABP enhancer; and the modified rabbit  $\beta$ -globin IVS, all upstream of the human  $\beta$ -domain deleted Factor VIII gene.

Plasmid pCY2-SE5 which was identical to pCY-2, except that the TBG promoter was replaced by the ET-1 gene promoter, and the ABP enhancers (both copies) were replaced by one copy of the SRE enhancer.

Plasmid pCY-201 which was identical to pCY-2, except that it lacked the 5' intron. Plasmid pCY-401 and pCY-402 which were identical to pCY-201, except that they contained one and two copies of the HBV PRE, respectively.

Expression levels for each of the foregoing gene constructs was compared in human hepatoma cells (HUH-7) maintained in DMEM (Dulbecco's modified Eagle medium (GIBCO BRL), supplemented with 10% heat inactivated fetal calf serum (10% FCS), penicillin (50 IU/ml), and streptomycin (50  $\mu$ g/ml) in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C. For experiments involving quantitation of human factor VIII protein, media was supplemented with an additional 10% FCS. DNA transfection was performed by a calcium phosphate coprecipitation method.

Other human Factor VIII gene constructs (shown below in Table I) tested for expression, prepared as described above, included constructs which were identical to pCY-2, except that they contained (a) the TBG promoter with no enhancer or 5' intron, (b) the TBG promoter with a 5' modified rabbit  $\beta$ -globin intron (present within the leader sequence), but no enhancer, (c) the TBG promoter with one copy of the ABP enhancer and a 5' modified rabbit  $\beta$ -globin intron (present within the leader sequence), and (d) the TBG promoter with two copies of the ABP enhancer and a 5' modified rabbit  $\beta$ -globin intron (present within the leader sequence).

Active Factor VIII protein was measured from tissue culture supernatants by COAtest VIII:c/4 kit assay specific for active Factor VIII protein. Transfection efficiencies were normalized to expression of cotransfected human growth hormone (hGH).

As shown below in Table I, liver-specific human Factor VIII expression is significantly increased by the combined use of the TBG promoter and a 5' intron within the 5' UTR of the gene construct. Expression is further increased (over 30 fold) by adding a copy of the ABP enhancer in the same construct. Expression is still further

increased (over 60 fold) by using two copies of the ABP enhancer in the same construct. In addition, as shown in Figure 18, expression is also significantly increased by adding one or more PRE sequences into the 3' UTR of the gene construct, although, in this experiment, not as much as by adding a 5' intron within the 5' UTR.

5

TABLE I

5' Region Tested	Fold Increase in Factor VIII Expression <i>In Vitro</i>
TBG Promoter	1
TBG Promoter, 5' Intron	3.5
ABP Enhancer (1 copy), TBG Promoter, 5' Intron	30.1
ABP Enhancer (2 copies), TBG Promoter, 5' Intron (pCY-2)	63.2

Expression of pCY2-SE5 was also tested and compared with pCY-2 in (a) bovine aortic endothelial cells and (b) HUH-7 cells. Transfections and Assays were performed as described above. Significantly more biologically active human Factor VIII was secreted from cells transfected with pCY2-SE5 than with pCY-2 (625 pg/ml vs. 280 pg/ml). While liver-specific pCY-2 expressed more than 10 ng/ml of human Factor VIII from HUH-7 cells, no human Factor VIII could be detected from pCY2-SE5 transfected HUH-7 cells.

Constructs were also tested *in vivo*. Specifically, pCY-2 and pCY2-SE5 were tested in mouse models by injecting mice (tail vein) with 10 µg of DNA in one 1.0 ml of solution (0.3 M NaCl, pH 9). Plasmids pCY-6, pLZ-6 and pLZ-6A (described in Example 1) were tested in the same experiment. Levels of human Factor VIII were measured in mouse serum. The results are shown in Figure 19. Plasmid pCY-2, containing the TBG promoter, 2 copies of the ABP enhancer, and an optimized 5' intron, had the highest expression, followed by pLZ-6A, pLZ-6, pCY2-SE5 and pCY-6.

Plasmid pCY-2 was also tested *in vivo* in mice, along with plasmid p25D which contained the same coding sequence (for human β-domain deleted Factor VIII) without an optimized 5' UTR. Specifically, instead of 2 copies of the ABP enhancer, one copy of the TBG promoter and a leader sequence containing an optimized (i.e., modified to contain consensus splice donor and acceptor sites and a consensus branch and pyrimidine track sequence) 5' rabbit β-globin intron (as contained in the 5' UTR of pCY-

2), p25D contained within its 5' UTR one copy of the CMV enhancer, one copy of the CMV promoter, and a leader sequence containing an unmodified short (130 bp) chimeric human IgE intron (containing uncorrected near consensus splice donor and acceptor sites). Plasmids were injected into mice (tail vein) in the form of

- 5 asialoorosomuroid/polylysine/DNA complexes formed as described below in Example 4. Mice were injected with 10 µg of DNA (complexed) in 1.0 of solution (0.3 M NaCl, pH 9).

The results are shown in Figure 25 and demonstrate that optimization of gene constructs by modification of 5' UTRs to contain novel combinations of strong tissue-specific promoters and enhancers, and optimized introns (e.g. modified to contain consensus splice donor and acceptor sites and a consensus branch and pyrimidine track sequence) significantly increases both levels and duration of gene expression. Notably, expression of p25D shut off after only 8 days, whereas expression of pCY-2 was maintained at nearly 100% of initial levels (well in the human therapeutic range of 10  
15 ng/ml or more) for over 10 days. In the same experiment, expression was maintained well in the therapeutic range for greater than 30 days.

Overall, the results of the foregoing examples demonstrate that gene expression can be significantly increased and prolonged *in vivo* by optimizing untranslated regulatory regions and/or coding sequences in accordance with the teachings of the  
20 present invention.

#### **EXAMPLE 4 - Targeted Delivery of Novel Genes to Cells**

Novel genes of the invention, such as novel Factor VIII genes contained in appropriate expression vectors, can be selectively delivered to target cells either *in vitro*  
25 or *in vivo* as follows:

#### **Formation of Targeted Molecular Complexes**

##### **I. Reagents**

Protamine, poly-L-lysine (4kD, 10kD, 26kD; mean MW) and ethidium bromide  
30 can be purchased from Sigma Chemical Co., St. Louis, MO. 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide (EDC) can be purchased from Aldrich Chemical Co, Milwaukee, WI. Synthetic polylysines can be purchased from Research Genetics (Huntsville, AL) or Dr. Schwabe (Protein Chemistry Facility at the Medical University of South Carolina). Orosomuroid (OR) can be purchased from Alpha Therapeutics, Los  
35 Angeles, CA. Asialoorosomuroid (AsOR) can be prepared from orosomuroid (15 mg/ml) by hydrolysis with 0.1 N sulfuric acid at 76°C for one hour. AsOR can then be purified from the reaction mixture by neutralization with 1.0 N NaOH to pH 5.5 and

exhaustive dialysis against water at room temperature. AsOR concentration can be determined using an extinction coefficient of  $0.92 \text{ ml mg}^{-1}, \text{ cm}^{-1}$  at 280 nm. The thiobarbituric acid assay of Warren (1959) *J. Biol. Chem.* 234:1971-1975 or of Uchida (1977) *J. Biochem.* 82:1425-1433 can be used to verify desialylation of the OR. AsOR prepared by the above method is typically 98% desialylated.

## II. Formation of Carrier Molecules

Carrier molecules capable of electrostatically binding to DNA can be prepared as follows: AsOR-poly-L-lysine conjugate (AP26K) can be formed by carbodiimide coupling similar to that reported by McKee (1994) *Bioconj. Chem.* 5:306-311. AsOR, 26kD poly-L-lysine and EDC in a 1:1:0.5 mass ratio can be reacted as follows. EDC (dry) is added directly to a stirring aqueous AsOR solution. Polylysine (26 kD) is then added, the reaction mixture adjusted to pH 5.5-6.0, and stirred for two hours at ambient temperature. The reaction can be quenched by addition of  $\text{Na}_3\text{PO}_4$  (200 mM, pH 11) to a final concentration of 10 mM. The AP26K conjugate can be first purified on a Fast Flow Q Sepharose anion exchange chromatography column (Pharmacia) eluted with 50 mM Tris, pH 7.5; and then dialyzed against water.

## III. Calculation of Charge Ratios (+/-)

Charge ratios of purified carrier molecules can be determined as follows: Protein-polylysine conjugates (e.g., AsOR-PL or OR-PL) are exhaustively dialyzed against ultra-pure water. An aliquot of the dialyzed conjugate solution is lyophilized, weighed and dissolved in ultra-pure water at a specific concentration (w/v). Since polylysine has minimal absorbance at 280 nm, the AsOR component of AsOR-polylysine (w/v) is calculated using the extinction coefficient at 280 nm. The composition of the conjugate is estimated by comparison of the concentration of the conjugate (w/v) with the concentration of AsOR (w/v) as determined by UV absorbance. The difference between the two determinations can be attributed to the polylysine component of the conjugate. The composition of OR-polylysine can be calculated in the same manner. The ratio of conjugate to DNA (w/w) necessary for specific charge ratios then can be calculated using the determined conjugate composition. Charge ratios for molecular complexes made with, e.g., polylysine or protamine, can be calculated from the amino acid composition.

## IV. Complexation With DNA

To form targeted DNA complexes, DNA (e.g., plasmid DNA) is preferably prepared in glycine (e.g., 0.44 M, pH 7), and is then rapidly added to an equal volume of carrier molecule, also in glycine (e.g., 0.44 M, pH 7), so that the final solution is isotonic.



### ***V. Fluorescence Quenching Assay***

Binding efficiencies of DNA to various polycationic carrier molecules can be examined using an ethidium bromide-based quenching assay. Solutions can be prepared containing 2.5 µg/ml EtBr and 10 µg/ml DNA (1:5 EtBr:DNA phosphates molar ratio) in a total volume of 1.0 ml. The polycation is added incrementally with fluorescence readings taken at each point using a fluorometer (e.g., a Sequoia-Turner 450), with excitation and emission wavelengths at 540 nm and 585 nm, respectively. Fluorescence readings are preferably adjusted to compensate for the change in volume due to the addition of polycation, if the polycation did not exceed 3% of the original volume. Results can be reported as the percentage of fluorescence relative to that of uncomplexed plasmid DNA (no polycation).

### **Cell Delivery *In Vivo* or *In Vitro***

DNA complexes prepared as described above can be administered in solution to subjects via injection. By way of example, a 0.1-1.0 ml dose of complex in solution can be injected intravenously via the tail vein into adult (e.g., 18-20 gm) BALB/C mice, at a dose ranging from <1.0-10.0 µg of DNA complex per mouse.

Alternatively, DNA complexes can be incubated with cells (e.g., HuH cells) in culture using any suitable transfection protocol known in the art for targeted uptake. Target cells for transfection must contain on their surface a component capable of binding to the cell-binding component of the DNA complex.

### **EQUIVALENTS**

Although the invention has been described with reference to its preferred embodiments, other embodiments can achieve the same results. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific embodiments described herein. Such equivalents are considered to be within the scope of this invention and are encompassed by the following claims.

### **INCORPORATION BY REFERENCE**

The contents of all references and patents cited herein are hereby incorporated by reference in their entirety.

**What is claimed is:**

1. An isolated DNA comprising one or more consensus or near consensus splice sites which have been corrected to increase expression of the DNA.  
5
2. The isolated DNA of claim 1 comprising a cDNA clone.
3. The isolated DNA of claim 1, wherein the one or more consensus or near consensus splice sites are corrected by conservative mutation of at least one consensus  
10 nucleotide.
4. The isolated DNA of claim 3, wherein the maximum number of conservative mutations are made within the one or more consensus or near consensus splice sites.  
15
5. The isolated DNA of claim 1 wherein the one or more consensus or near consensus splice sites comprises a 5' splice donor site which is corrected by mutating one or both of the nucleotides within the essential GT pair.
- 20 6. The isolated DNA of claim 1 wherein the one or more consensus or near consensus splice sites comprises a 3' splice acceptor site which is corrected by mutating one or both of the nucleotides within the essential AG pair.
7. The isolated DNA of claim 1 comprising a nucleotide sequence which  
25 encodes a Factor VIII protein.
8. The isolated DNA of claim 1 comprising a cDNA which is expressed as a  $\beta$ -domain deleted Factor VIII protein.
- 30 9. The isolated DNA of claim 8 comprising the nucleotide sequence shown in SEQ ID NO:1.
10. The isolated DNA of claim 1 comprising the coding region of a full-length Factor VIII gene, wherein the coding region contains an intron spanning all or a  
35 portion of the gene encoding the  $\beta$ -domain.

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11. The isolated DNA of claim 8 further comprising a second intron upstream of the coding region.

12 An isolated DNA comprising the coding region of a full-length Factor VIII gene, wherein the coding region contains an intron spanning the portion of the gene encoding the  $\beta$ -domain.

13 The isolated DNA of claim 12 comprising the coding region of the nucleotide sequence shown in SEQ ID NO:3.

10

14. The isolated DNA of claim 12 further comprising one or more consensus or near consensus splice sites which have been corrected.

15 An isolated DNA which is expressed as a  $\beta$ -domain deleted Factor VIII protein, said DNA comprising the coding region of a full-length Factor VIII gene modified to (a) correct one or more consensus or near consensus splice sites within the coding region and (b) to incorporate an intron into the coding region which spans the portion of the gene encoding the  $\beta$ -domain.

16. The isolated DNA of claim 15 which encodes a human  $\beta$ -domain deleted Factor VIII protein.

17. An expression vector comprising the isolated DNA of claim 1 operably linked to a promoter sequence.

25

18. An expression vector comprising the isolated DNA of claim 7 operably linked to a promoter sequence.

19. An expression vector comprising the isolated DNA of claim 10 operably linked to a promoter sequence.

30

20. An expression vector comprising the isolated DNA of claim 12 operably linked to a promoter sequence.

21. A molecular complex comprising the expression vector of claim 17 linked to an agent which binds to a component on the surface of a mammalian cell.

35

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22. A molecular complex comprising the expression vector of claim 18 linked to an agent which binds to a component on the surface of a mammalian cell.

23. A molecular complex comprising the expression vector of claim 19  
5 linked to an agent which binds to a component on the surface of a mammalian cell.

24. A molecular complex comprising the expression vector of claim 20 linked to an agent which binds to a component on the surface of a mammalian cell.

10 25. A method of increasing expression of a gene comprising correcting one or more consensus or near consensus splice sites within the nucleotide sequence of the gene.

26. The method of claim 25 wherein the step of correcting the one or more  
15 consensus or near consensus splice sites comprises conservatively mutating one or more consensus nucleotides within the consensus or near consensus splice site.

27. The method of claim 25 wherein the step of correcting the one or more  
20 consensus or near consensus splice sites comprises making the maximum number of conservative mutations possible to consensus nucleotides within the consensus or near consensus splice site.

28. The method of claim 25 comprising mutating one or both of the  
nucleotides within the essential GT pair, if the consensus or near consensus splice site is  
25 a 5' splice site, or mutating one or both of the nucleotides within the essential AG pair,  
if the consensus or near consensus splice site is a 3' splice site.

29. The method of claim 28 wherein the gene encodes a Factor VIII protein.

30 30. The method of claim 25 wherein the gene is expressed as a  $\beta$ -domain  
deleted Factor VIII protein.

31. The method of claim 30 wherein the gene comprises the nucleotide  
sequence shown in SEQ ID NO:1.  
35

32. The method of claim 25 wherein the gene comprises the coding region of a full-length Factor VIII gene, and the method further comprises the step of inserting an intron into the coding region of the gene so that the intron spans all or a portion of the segment of the gene encoding the  $\beta$ -domain.

5

33. The method of claim 32 further comprising inserting a second intron upstream of the coding region of the gene.

34. A method of increasing expression of a gene encoding Factor VIII comprising inserting into the coding region of the gene an intron which spans all or a portion of the portion of the gene encoding the  $\beta$ -domain.

10

35. The method of claim 34 further comprising correcting one or more consensus or near consensus splice sites within the Factor VIII gene by conservative mutation of a consensus nucleotide.

15

36. A method of increasing expression of a gene encoding Factor VIII comprising correcting one or more consensus or near consensus splice sites within the gene.

20

37. The method of claim 36 wherein the correction is made by conservative mutation of a consensus nucleotide located within the coding region of the gene.

38. A method of producing Factor VIII comprising introducing the expression vector of claim 19 into a host cell capable of expressing the vector, and allowing for expression of the vector.

25

39. A method of producing Factor VIII comprising introducing the expression vector of claim 20 into a host cell capable of expressing the vector, and allowing for expression of the vector.

30

40. An expression vector comprising a liver-specific promoter and a liver-specific enhancer, said promoter and enhancer being derived from different genes.

41. The expression vector of claim 40, wherein the promoter and enhancer are located upstream from the coding sequence of a gene.

35

- 48 -

42. The expression vector of claim 41, wherein the coding sequence is expressed as a  $\beta$ -domain deleted human Factor VIII protein.

43. The expression vector of claim 40, wherein the liver-specific promoter is  
5 the human thyroid binding globulin promoter.

44. The expression vector of claim 40, wherein the liver-specific enhancer is the alpha-1 microglobulin/bikunin enhancer.

10 45. The expression vector of claim 41 further comprising one or more introns located (a) downstream from the promoter and enhancer and (b) upstream from the coding sequence.

46. The expression vector of claim 45, wherein the intron is located within  
15 the leader sequence of the gene.

47. The expression vector of claim 45, wherein the intron comprises one or more consensus splice sites.

20 48. The expression vector of claim 46, wherein the leader sequence has no secondary structure when transcribed as RNA.

49. The expression vector of claim 41, wherein the 3' untranslated region of the gene is modified to increase processing, export or stability of the mRNA transcribed  
25 from the gene.

50. An expression vector comprising the human thyroid binding globulin promoter and the alpha-1 microglobulin/bikunin enhancer.

30 51. The expression vector of claim 50 comprising two or more copies of the alpha-1 microglobulin/bikunin enhancer.

52. The expression vector of claim 50, wherein the human thyroid binding globulin promoter and the alpha-1 microglobulin/bikunin enhancer are located upstream  
35 from the coding sequence of a gene.

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53. The expression vector of claim 52, wherein the coding sequence is also preceded upstream by a leader sequence comprising one or more introns.

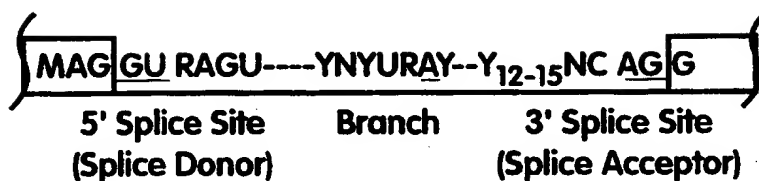
54. The expression vector of claim 51 wherein the coding sequence is  
5 expressed as a  $\beta$ -domain deleted human Factor VIII protein.

55. The expression vector of claim 53, wherein the intron comprises a consensus 5' splice donor site, and a consensus 3' splice acceptor site.

10 56. The expression vector of claim 53, wherein the intron has no secondary structure when transcribed as RNA.

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## Anatomy of an Intron



M = C or A

R = puRine (A or G)

Y = pYrimidine (C or T or U)

Fig.1

Conservative Mutagenesis of  
Near Consensus 3' Splice sequence

Conserved Splice  
Acceptor sequence

TTC TCA GGA GAA  
Phe SER Gly Glu

Alternate Codons	
Ser	Gly
TCC	None
TCG	
TCT	

Fig. 2



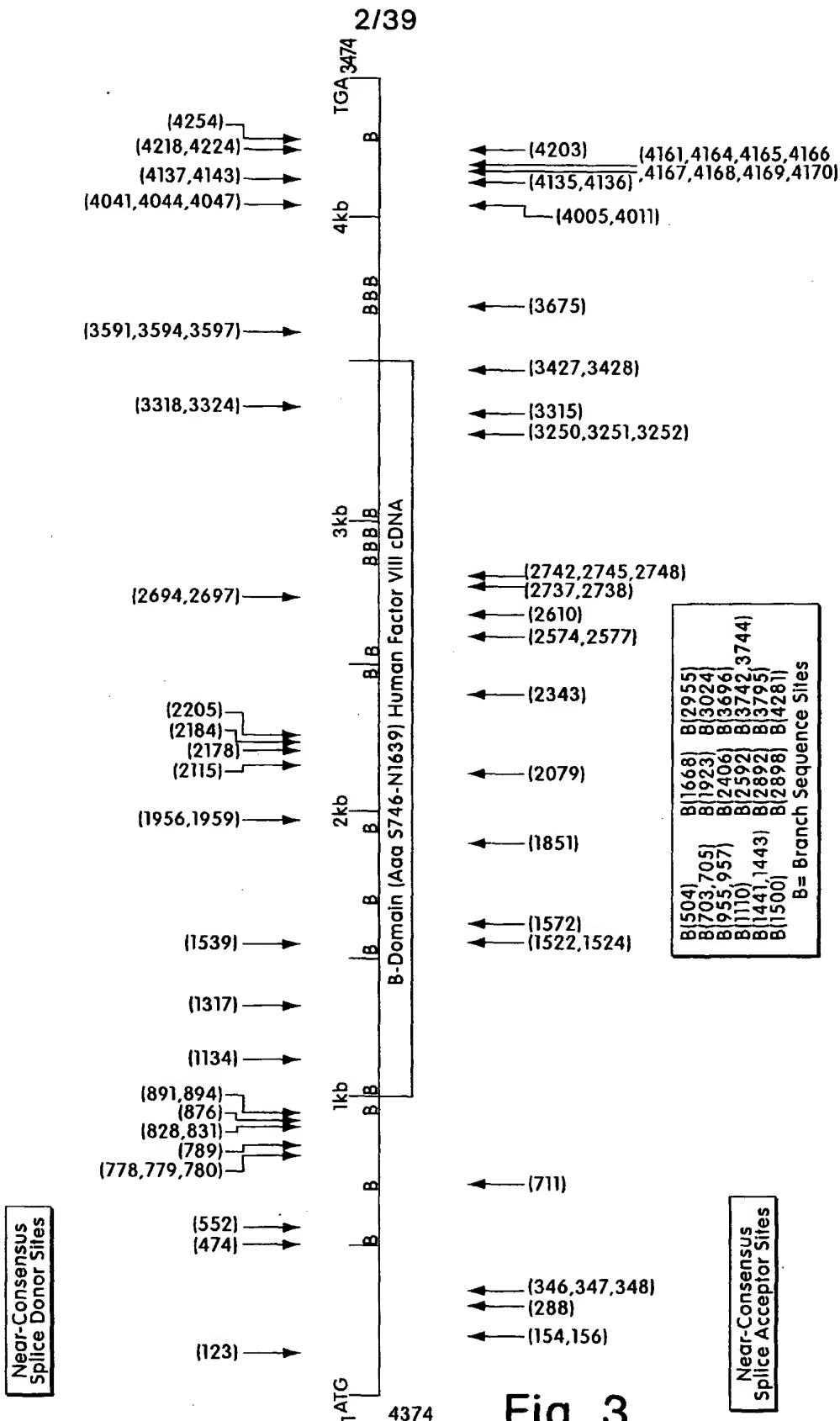


Fig. 3

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## NC-Splice Donor Changes:

Location in Bayer BDD-Factor VIII cDNA	Nucleotide Change	Codon	Amino Acid
123	T → A	GGA	G
474	G A	CAA	Q
552	T C	GTT	V
778	T A	AGC	S
779	C G	AGC	S
780	T C	AGC	S
789	T A	GGA	G
828	G T	GTT	V
831	T A	ATA	I
876	T A	GGA	G
891	G T	GTT	V
894	G A	AGA	R
1134	G A	AGA	R
1317	T A	GGA	G
1539	T A	GGA	G
1956	G A	GAA	E
1959	G A	GTA	V
2115	T A	GGA	G
2178	G A	AAA	K
2184	T C	TCC	S
2205	T A	GGA	G
2694	A T	GTT	V
2697	T C	ACC	T
3318	T A	GGA	G
3324	T C	TTC	F
3591	G A	AAA	K
3594	G T	GTT	V
3597	T C	GAC	D
4041	G A	CAA	Q
4044	G T	GTT	V
4047	T C	AAC	N
4137	C T	TCT	S
4143	T C	TAC	Y
4218	G A	AAA	K
4224	T C	TTC	F
4254	G C	GTC	V

Fig. 4A

## NC-Splice Acceptor Changes

Location in Bayer BDD-Factor VIII cDNA	Nucleotide change	Codon	Amino Acid
154	A→C	CGC	R
156	A	CGC	R
288	G	CAA	Q
346	A	TCC	S
347	G	TCC	S
348	T	TCC	S
711	G	CAA	Q
1522	A	CGC	R
1524	G	CGC	R
1572	A	CCC	P
1851	A	CCC	P
2079	A	TCC	S
2343	G	CAA	Q
2574	T	GTA	V
2577	T	GTA	V
2610	G	CAA	Q
2737	A	TCC	S
2738	G	TCC	S
2742	T	CTC	L
2745	T	ATA	I
2748	T	TCA	S
3250	A	TCC	S
3251	G	TCC	S
3252	T	TCC	S
3315	A	CCC	P
3427	A	TCC	S
3428	G	TCC	S
3675	G	CAA	Q
4005	T	CTA	L
4011	C	CTA	L
4135	A	TCT	S
4136	G	TCC	S
4161	C	ATA	I
4164	C	TCG	S
4165	A	TCG	S
4166	G	TCG	S
4167	C	TCG	S
4168	A	TCG	S
4169	G	TCG	S
4170	T	TCG	S
4203	G	CAA	Q

Fig. 4B

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Branch (lariat) sequence changes:

Location in Bayer BDD-Factor VIII cDNA	Nucleotide change	Codon	Amino Acid
504	T → C	TCC	S
703	T	CTC	L
705	G	CTC	L
955	T	CTC	L
957	G	CTC	L
1110	T	ACC	T
1441	T	CTC	L
1443	G	CTC	L
1500	T	ACC	T
1668	T	GTC	V
1923	T	TCC	S
2406	T	TCC	S
2592	T	ACC	T
2892	T	TCC	S
2898	T	GTC	V
2955	T	ACC	T
3024	T	TCC	S
3696	T	CTC	L
3742	T	CTC	L
3744	A	CTC	L
3795	T	TTC	F
4281	G	CTC	L

Fig. 4C

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	5	10	15	20	25	30	35	40	45							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	ATG	GAA	ATA	GAG	CTC	TCC	ACC	TGC	TTC	TTT	CTG	TGC	CTT	TTG	CGA	TTC
1. p25Dcod				10			20			30				40		
( 16902 )	ATG	GAA	ATA	GAG	CTC	TCC	ACC	TGC	TTC	TTT	CTG	TGC	CTT	TTG	CGA	TTC>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	ATG	GAA	ATA	GAG	CTC	TCC	ACC	TGC	TTC	TTT	CTG	TGC	CTT	TTG	CGA	TTC
	50	55	60	65	70	75	80	85	90	95						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	TGC	TTT	AGT	GCC	ACC	AGA	AGA	TAC	TAC	CTG	GGT	GCA	GTG	GAA	CTG	TCA
1. p25Dcod	50			60			70			80			90			
( 16902 )	TGC	TTT	AGT	GCC	ACC	AGA	AGA	TAC	TAC	CTG	GGT	GCA	GTG	GAA	CTG	TCA>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	TGC	TTT	AGT	GCC	ACC	AGA	AGA	TAC	TAC	CTG	GGT	GCA	GTG	GAA	CTG	TCA
	100	105	110	115	120	125	130	135	140							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	TGG	GAC	TAT	ATG	CAA	AGT	GAT	CTC	GGA	GAG	CTG	CCT	GTG	GAC	GCA	AGA
1. p25Dcod	100			110			120			130			140			
( 16902 )	TGG	GAC	TAT	ATG	CAA	AGT	GAT	CTC	GGA	GAG	CTG	CCT	GTG	GAC	GCA	AGA>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	TGG	GAC	TAT	ATG	CAA	AGT	GAT	CTC	GGA	GAG	CTG	CCT	GTG	GAC	GCA	AGA
	145	150	155	160	165	170	175	180	185	190						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	TTT	CCT	CCT	CGC	GTG	CCA	AAA	TCT	TTT	CCA	TTC	AAC	ACC	TCA	GTC	GTG
1. P25Dcod	150			160			170			180			190			
( 16902 )	TTT	CCT	CCT	aGa	GTG	CCA	AAA	TCT	TTT	CCA	TTC	AAC	ACC	TCA	GTC	GTG>
	AAA	AAA	AAA	v^v	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	TTT	CCT	CCT	CGC	GTG	CCA	AAA	TCT	TTT	CCA	TTC	AAC	ACC	TCA	GTC	GTG
	195	200	205	210	215	220	225	230	235	240						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	TAC	AAA	AAG	ACT	CTG	TTT	GTA	GAA	TTC	ACG	GTT	CAC	CTT	TTC	AAC	ATC
1. p25Dcod	200			210			220			230			240			
( 16902 )	TAC	AAA	AAG	ACT	CTG	TTT	GTA	GAA	TTC	ACG	GTT	CAC	CTT	TTC	AAC	ATC>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	TAC	AAA	AAG	ACT	CTG	TTT	GTA	GAA	TTC	ACG	GTT	CAC	CTT	TTC	AAC	ATC

Fig. 5A

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	245	250	255	260	265	270	275	280	285							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	GCT	AAG	CCA	AGG	CCA	CCC	TGG	ATG	GGT	CTG	CTA	GGT	CCT	ACC	ATC	CAA
1. p25Dcod			250		260		270		280							
( 16902 )	GCT	AAG	CCA	AGG	CCA	CCC	TGG	ATG	GGT	CTG	CTA	GGT	CCT	ACC	ATC	CAG>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAV
pDJCcoding	GCT	AAG	CCA	AGG	CCA	CCC	TGG	ATG	GGT	CTG	CTA	GGT	CCT	ACC	ATC	CAA

	290	295	300	305	310	315	320	325	330	335						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	GCT	GAG	GTT	TAT	GAT	ACA	GTG	GTC	ATT	ACA	CTT	AAG	AAC	ATG	GCT	TCC
1. p25Dco	290		300		310		320		330							
( 16902 )	GCT	GAG	GTT	TAT	GAT	ACA	GTG	GTC	ATT	ACA	CTT	AAG	AAC	ATG	GCT	TCC>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	GCT	GAG	GTT	TAT	GAT	ACA	GTG	GTC	ATT	ACA	CTT	AAG	AAC	ATG	GCT	TCC

	340	345	350	355	360	365	370	375	380							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	CAT	CCT	GTC	TCC	CTT	CAT	GCT	GTT	GGT	GTA	TCC	TAC	TGG	AAA	GCT	TCT
1. p25Dcod	340		350		360		370		380							
( 16902 )	CAT	CCT	GTC	agt	CTT	CAT	GCT	GTT	GGT	GTA	TCC	TAC	TGG	AAA	GCT	TCT>
	AAA	AAA	AAA	vvv	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	CAT	CCT	GTC	TCC	CTT	CAT	GCT	GTT	GGT	GTA	TCC	TAC	TGG	AAA	GCT	TCT

	385	390	395	400	405	410	415	420	425	430						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	GAG	GGA	GCT	GAA	TAT	GAT	GAT	CAG	ACC	AGT	CAA	AGG	GAG	AAA	GAA	GAT
1. p25Dcod		390		400		410		420		430						
( 16902 )	GAG	GGA	GCT	GAA	TAT	GAT	GAT	CAG	ACC	AGT	CAA	AGG	GAG	AAA	GAA	GAT>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	GAG	GGA	GCT	GAA	TAT	GAT	GAT	CAG	ACC	AGT	CAA	AGG	GAG	AAA	GAA	GAT

	435	440	445	450	455	460	465	470	475	480						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	GAT	AAA	GTC	TTC	CCT	GGT	GGA	AGC	CAT	ACA	TAT	GTC	TGG	CAA	GTC	CTG
1. p25Dcod		440		450		460		470		480						
( 16902 )	GAT	AAA	GTC	TTC	CCT	GGT	GGA	AGC	CAT	ACA	TAT	GTC	TGG	CAG	GTC	CTG>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAV	AAA	AAA
pDJCcoding	GAT	AAA	GTC	TTC	CCT	GGT	GGA	AGC	CAT	ACA	TAT	GTC	TGG	CAA	GTC	CTG

	485	490	495	500	505	510	515	520	525							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	AAA	GAG	AAT	GGT	CCA	ATG	GCC	TCC	GAC	CCA	CTG	TGC	CTT	ACC	TAC	TCA
1. p25Dcod		490		500		510		520								
( 16902 )	AAA	GAG	AAT	GGT	CCA	ATG	GCC	TCC	GAC	CCA	CTG	TGC	CTT	ACC	TAC	TCA>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAV	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	AAA	GAG	AAT	GGT	CCA	ATG	GCC	TCC	GAC	CCA	CTG	TGC	CTT	ACC	TAC	TCA

Fig. 5B

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	530	535	540	545	550	555	560	565	570	575						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	TAT	CTT	TCT	CAT	GTG	GAC	CTG	GTT	AAA	GAC	TTG	AAT	TCA	GGC	CTC	ATT
1. p25Dco	530		540		550		560		570							
( 16902 )	TAT	CTT	TCT	CAT	GTG	GAC	CTG	GTA	AAA	GAC	TTG	AAT	TCA	GGC	CTC	ATT>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	TAT	CTT	TCT	CAT	GTG	GAC	CTG	GTT	AAA	GAC	TTG	AAT	TCA	GGC	CTC	ATT

	580	585	590	595	600	605	610	615	620							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	GGA	GCC	CTA	CTA	GTA	TGT	AGA	GAA	GGG	AGT	CTG	GCC	AAG	GAA	AAG	ACA
1. p25 Dcod	580		590		600		610		620							
( 16902 )	GGA	GCC	CTA	CTA	GTA	TGT	AGA	GAA	GGG	AGT	CTG	GCC	AAG	GAA	AAG	ACA>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	GGA	GCC	CTA	CTA	GTA	TGT	AGA	GAA	GGG	AGT	CTG	GCC	AAG	GAA	AAG	ACA

	625	630	635	640	645	650	655	660	665	670						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	CAG	ACC	TTG	CAC	AAA	TTT	ATA	CTA	CTT	TTT	GCT	GTA	TTT	GAT	GAA	GGG
1. p25Dcod		630		640		650		660		670						
( 16902 )	CAG	ACC	TTG	CAC	AAA	TTT	ATA	CTA	CTT	TTT	GCT	GTA	TTT	GAT	GAA	GGG>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	CAG	ACC	TTG	CAC	AAA	TTT	ATA	CTA	CTT	TTT	GCT	GTA	TTT	GAT	GAA	GGG

	675	680	685	690	695	700	705	710	715	720						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	AAA	AGT	TGG	CAC	TCA	GAA	ACA	AAG	AAC	TCC	CTC	ATG	CAA	GAT	AGG	GAT
1. p25Dcod		680		690		700		710		720						
( 16902 )	AAA	AGT	TGG	CAC	TCA	GAA	ACA	AAG	AAC	TCC	tTg	ATG	CAG	GAT	AGG	GAT>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	AAA	AGT	TGG	CAC	TCA	GAA	ACA	AAG	AAC	TCC	CTC	ATG	CAA	GAT	AGG	GAT

	725	730	735	740	745	750	755	760	765							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	GCT	GCA	TCT	GCT	CGG	GCC	TGG	CCT	AAA	ATG	CAC	ACA	GTC	AAT	GGT	TAT
1. p25 Dcod		730		740		750		760								
( 16902 )	GCT	GCA	TCT	GCT	CGG	GCC	TGG	CCT	AAA	ATG	CAC	ACA	GTC	AAT	GGT	TAT>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	GCT	GCA	TCT	GCT	CGG	GCC	TGG	CCT	AAA	ATG	CAC	ACA	GTC	AAT	GGT	TAT

	770	775	780	785	790	795	800	805	810	815						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	GTA	AAC	AGG	AGC	CTG	CCA	GGA	CTG	ATT	GGA	TGC	CAC	AGG	AAA	TCA	GTC
( 16902 )	770		780		790		800		810							
	GTA	AAC	AGG	tct	CTG	CCA	GGt	CTG	ATT	GGA	TGC	CAC	AGG	AAA	TCA	GTC>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	GTA	AAC	AGG	AGC	CTG	CCA	GGA	CTG	ATT	GGA	TGC	CAC	AGG	AAA	TCA	GTC

Fig. 5C

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	820	825	830	835	840	845	850	855	860							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	TAT	TGG	CAT	GTT	ATA	GGA	ATG	GGC	ACC	ACT	CCT	GAA	GTG	CAC	TCA	ATA
1. p25Dcod	820				830				840				850			860
( 16902 )	TAT	TGG	CAT	GTg	ATt	GGA	ATG	GGC	ACC	ACT	CCT	GAA	GTG	CAC	TCA	ATA>
	AAA	AAA	AAA	AAv	AAv	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	TAT	TGG	CAT	GTT	ATA	GGA	ATG	GGC	ACC	ACT	CCT	GAA	GTG	CAC	TCA	ATA

	865	870	875	880	885	890	895	900	905	910						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	TTC	CTC	GAA	GGA	CAC	ACA	TTT	CTT	GTT	AGA	AAC	CAT	CGC	CAG	GCG	TCC
1. p25Dcod	870				880				890				900			910
( 16902 )	TTC	CTC	GAA	GGt	CAC	ACA	TTT	CTT	GTg	AGg	AAC	CAT	CGC	CAG	GCG	TCC>
	AAA	AAA	AAA	AAv	AAA	AAA	AAA	AAA	AAv	AAv	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	TTC	CTC	GAA	GGA	CAC	ACA	TTT	CTT	GTT	AGA	AAC	CAT	CGC	CAG	GCG	TCC

	915	920	925	930	935	940	945	950	955	960						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	TTG	GAA	ATC	TCG	CCA	ATA	ACT	TTC	CTT	ACT	GCT	CAA	ACA	CTC	CTC	ATG
( 16902 )	920				930				940				950			960
	TTG	GAA	ATC	TCG	CCA	ATA	ACT	TTC	CTT	ACT	GCT	CAA	ACA	CTC	tTg	ATG>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAv	AAA
pDJCcoding	TTG	GAA	ATC	TCG	CCA	ATA	ACT	TTC	CTT	ACT	GCT	CAA	ACA	CTC	CTC	ATG

	965	970	975	980	985	990	995	1000	1005							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	GAC	CTT	GGA	CAG	TTT	CTA	CTG	TTT	TGT	CAT	ATC	TCT	TCC	CAC	CAA	CAT
1. p25Dcod	970				980				990				1000			
( 16902 )	GAC	CTT	GGA	CAG	TTT	CTA	CTG	TTT	TGT	CAT	ATC	TCT	TCC	CAC	CAA	CAT>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	GAC	CTT	GGA	CAG	TTT	CTA	CTG	TTT	TGT	CAT	ATC	TCT	TCC	CAC	CAA	CAT

	1010	1015	1020	1025	1030	1035	1040	1045	1050	1055						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	GAT	GGC	ATG	GAA	GCT	TAT	GTC	AAA	GTA	GAC	AGC	TGT	CCA	GAG	GAA	CCC
1. p25Dc	1010				1020				1030				1040			1050
( 16902 )	GAT	GGC	ATG	GAA	GCT	TAT	GTC	AAA	GTA	GAC	AGC	TGT	CCA	GAG	GAA	CCC>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	GAT	GGC	ATG	GAA	GCT	TAT	GTC	AAA	GTA	GAC	AGC	TGT	CCA	GAG	GAA	CCC

	1060	1065	1070	1075	1080	1085	1090	1095	1100							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	CAA	CTA	CGA	ATG	AAA	AAT	AAT	GAA	GAA	GCG	GAA	GAC	TAT	GAT	GAT	GAT
1. p25Dcod	1060				1070				1080				1090			1100
( 16902 )	CAA	CTA	CGA	ATG	AAA	AAT	AAT	GAA	GAA	GCG	GAA	GAC	TAT	GAT	GAT	GAT>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	CAA	CTA	CGA	ATG	AAA	AAT	AAT	GAA	GAA	GCG	GAA	GAC	TAT	GAT	GAT	GAT

Fig. 5D

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	1105	1110	1115	1120	1125	1130	1135	1140	1145	1150						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	CTT	ACC	GAT	TCT	GAA	ATG	GAT	GTG	GTC	AGA	TTT	GAT	GAT	GAC	AAC	TCT
1. p25Dcod		1110		1120		1130		1140		1150						
( 16902 )	CTT	ACT	GAT	TCT	GAA	ATG	GAT	GTG	GTC	AGg	TTT	GAT	GAT	GAC	AAC	TCT>
	AAA	AAV	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAV	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	CTT	ACC	GAT	TCT	GAA	ATG	GAT	GTG	GTC	AGA	TTT	GAT	GAT	GAC	AAC	TCT

	1155	1160	1165	1170	1175	1180	1185	1190	1195	1200						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	CCT	TCC	TTT	ATC	CAA	ATT	CGC	TCA	GTT	GCC	AAG	AAG	CAT	CCT	AAA	ACT
1. p25Dcod		1160		1170		1180		1190		1200						
( 16902 )	CCT	TCC	TTT	ATC	CAA	ATT	CGC	TCA	GTT	GCC	AAG	AAG	CAT	CCT	AAA	ACT>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	CCT	TCC	TTT	ATC	CAA	ATT	CGC	TCA	GTT	GCC	AAG	AAG	CAT	CCT	AAA	ACT

	1205	1210	1215	1220	1225	1230	1235	1240	1245							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	TGG	GTA	CAT	TAC	ATT	GCT	GCT	GAA	GAG	GAG	GAC	TGG	GAC	TAT	GCT	CCC
1. p25Dcod		1210		1220		1230		1240								
( 16902 )	TGG	GTA	CAT	TAC	ATT	GCT	GCT	GAA	GAG	GAG	GAC	TGG	GAC	TAT	GCT	CCC>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	TGG	GTA	CAT	TAC	ATT	GCT	GCT	GAA	GAG	GAG	GAC	TGG	GAC	TAT	GCT	CCC

	1250	1255	1260	1265	1270	1275	1280	1285	1290	1295						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	TTA	GTC	CTC	GCC	CCC	GAT	GAC	AGA	AGT	TAT	AAA	AGT	CAA	TAT	TTG	AAC
1. p25Dc	1250		1260		1270		1280		1290							
( 16902 )	TTA	GTC	CTC	GCC	CCC	GAT	GAC	AGA	AGT	TAT	AAA	AGT	CAA	TAT	TTG	AAC>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	TTA	GTC	CTC	GCC	CCC	GAT	GAC	AGA	AGT	TAT	AAA	AGT	CAA	TAT	TTG	AAC

	1300	1305	1310	1315	1320	1325	1330	1335	1340							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	AAT	GGC	CCT	CAG	CGG	ATT	GGA	AGG	AAG	TAC	AAA	AAA	GTC	CGA	TTT	ATG
1. P25Dcod	1300		1310		1320		1330		1340							
( 16902 )	AAT	GGC	CCT	CAG	CGG	ATT	GGA	AGG	AAG	TAC	AAA	AAA	GTC	CGA	TTT	ATG>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	AAT	GGC	CCT	CAG	CGG	ATT	GGA	AGG	AAG	TAC	AAA	AAA	GTC	CGA	TTT	ATG

	1345	1350	1355	1360	1365	1370	1375	1380	1385	1390						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	GCA	TAC	ACA	GAT	GAA	ACC	TTT	AAG	ACT	CGT	GAA	GCT	ATT	CAG	CAT	GAA
1. p25Dcod		1350		1360		1370		1380		1390						
( 16902 )	GCA	TAC	ACA	GAT	GAA	ACC	TTT	AAG	ACT	CGT	GAA	GCT	ATT	CAG	CAT	GAA>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	GCA	TAC	ACA	GAT	GAA	ACC	TTT	AAG	ACT	CGT	GAA	GCT	ATT	CAG	CAT	GAA

Fig. 5E

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	1395	1400	1405	1410	1415	1420	1425	1430	1435	1440						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	TCA	GGA	ATC	TTG	GGA	CCT	TTA	CTT	TAT	GGG	GAA	GTT	GGA	GAC	ACA	CTG
1. p25 Dcod		1400		1410		1420		1430		1440						
( 16902 )	TCA	GGA	ATC	TTG	GGA	CCT	TTA	CTT	TAT	GGG	GAA	GTT	GGA	GAC	ACA	CTG>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	TCA	GGA	ATC	TTG	GGA	CCT	TTA	CTT	TAT	GGG	GAA	GTT	GGA	GAC	ACA	CTG

	1445	1450	1455	1460	1465	1470	1475	1480	1485							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	CTC	ATT	ATA	TTT	AAG	AAT	CAA	GCA	AGC	AGA	CCA	TAT	AAC	ATC	TAC	CCT
1. P25Dcod		1450		1460		1470		1480								
( 16902 )	tTg	ATT	ATA	TTT	AAG	AAT	CAA	GCA	AGC	AGA	CCA	TAT	AAC	ATC	TAC	CCT>
	v^v	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	CTC	ATT	ATA	TTT	AAG	AAT	CAA	GCA	AGC	AGA	CCA	TAT	AAC	ATC	TAC	CCT

	1490	1495	1500	1505	1510	1515	1520	1525	1530	1535						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	CAC	GGA	ATC	ACC	GAT	GTC	CGT	CCT	TTG	TAT	TCA	CGC	AGA	TTA	CCA	AAA
1. p25Dc	1490		1500		1510		1520		1530							
( 16902 )	CAC	GGA	ATC	ACt	GAT	GTC	CGT	CCT	TTG	TAT	TCA	aGg	AGA	TTA	CCA	AAA>
	AAA	AAA	AAA	AAv	AAA	AAA	AAA	AAA	AAA	AAA	AAA	v^v	AAA	AAA	AAA	AAA
pDJCcoding	CAC	GGA	ATC	ACC	GAT	GTC	CGT	CCT	TTG	TAT	TCA	CGC	AGA	TTA	CCA	AAA

	1540	1545	1550	1555	1560	1565	1570	1575	1580							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	GGA	GTA	AAA	CAT	TTG	AAG	GAT	TTT	CCA	ATT	CTG	CCC	GGA	GAA	ATA	TTC
1. p25Dcod	1540		1550		1560		1570		1580							
( 16902 )	GGt	GTA	AAA	CAT	TTG	AAG	GAT	TTT	CCA	ATT	CTG	CCa	GGA	GAA	ATA	TTC>
	AAv	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAv	AAA	AAA	AAA	AAA
pDJCcoding	GGA	GTA	AAA	CAT	TTG	AAG	GAT	TTT	CCA	ATT	CTG	CCC	GGA	GAA	ATA	TTC

	1585	1590	1595	1600	1605	1610	1615	1620	1625	1630						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	AAA	TAT	AAA	TGG	ACA	GTG	ACT	GTA	GAA	GAT	GGG	CCA	ACT	AAA	TCA	GAT
1. p25Dcod	1590		1600		1610		1620		1630							
( 16902 )	AAA	TAT	AAA	TGG	ACA	GTG	ACT	GTA	GAA	GAT	GGG	CCA	ACT	AAA	TCA	GAT>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	AAA	TAT	AAA	TGG	ACA	GTG	ACT	GTA	GAA	GAT	GGG	CCA	ACT	AAA	TCA	GAT

	1635	1640	1645	1650	1655	1660	1665	1670	1675	1680						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	CCT	CGG	TGC	CTG	ACC	CGC	TAT	TAC	TCT	AGT	TTC	GTC	AAT	ATG	GAG	AGA
1. p25Dcod	1640		1650		1660		1670		1680							
( 16902 )	CCT	CGG	TGC	CTG	ACC	CGC	TAT	TAC	TCT	AGT	TTC	Gt	AAT	ATG	GAG	AGA>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAv	AAA	AAA	AAA	AAA
pDJCcoding	CCT	CGG	TGC	CTG	ACC	CGC	TAT	TAC	TCT	AGT	TTC	GTC	AAT	ATG	GAG	AGA

Fig. 5F

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	1685	1690	1695	1700	1705	1710	1715	1720	1725							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	GAT	CTA	GCT	TCA	GGA	CTC	ATT	GGC	CCT	CTC	CTC	ATC	TGC	TAC	AAA	GAA
1. p25Dcod		1690		1700		1710		1720								
( 16902 )	GAT	CTA	GCT	TCA	GGA	CTC	ATT	GGC	CCT	CTC	CTC	ATC	TGC	TAC	AAA	GAA>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	GAT	CTA	GCT	TCA	GGA	CTC	ATT	GGC	CCT	CTC	CTC	ATC	TGC	TAC	AAA	GAA

	1730	1735	1740	1745	1750	1755	1760	1765	1770	1775						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	TCT	GTA	GAT	CAA	AGA	GGA	AAC	CAG	ATA	ATG	TCA	GAC	AAG	AGG	AAT	GTC
1. p25Dc	1730		1740		1750		1760		1770							
( 16902 )	TCT	GTA	GAT	CAA	AGA	GGA	AAC	CAG	ATA	ATG	TCA	GAC	AAG	AGG	AAT	GTC>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	TCT	GTA	GAT	CAA	AGA	GGA	AAC	CAG	ATA	ATG	TCA	GAC	AAG	AGG	AAT	GTC

	1780	1785	1790	1795	1800	1805	1810	1815	1820							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	ATC	CTG	TTT	TCT	GTA	TTT	GAT	GAG	AAC	CGA	AGC	TGG	TAC	CTC	ACA	GAG
1. p25Dcod	1780		1790		1800		1810		1820							
( 16902 )	ATC	CTG	TTT	TCT	GTA	TTT	GAT	GAG	AAC	CGA	AGC	TGG	TAC	CTC	ACA	GAG>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	ATC	CTG	TTT	TCT	GTA	TTT	GAT	GAG	AAC	CGA	AGC	TGG	TAC	CTC	ACA	GAG

	1825	1830	1835	1840	1845	1850	1855	1860	1865	1870						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	AAT	ATA	CAA	CGC	TTT	CTC	CCC	AAT	CCC	GCT	GGA	GTG	CAG	CTT	GAG	GAT
1. p25Dcod		1830		1840		1850		1860		1870						
( 16902 )	AAT	ATA	CAA	CGC	TTT	CTC	CCC	AAT	CCa	GCT	GGA	GTG	CAG	CTT	GAG	GAT>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAv	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	AAT	ATA	CAA	CGC	TTT	CTC	CCC	AAT	CCC	GCT	GGA	GTG	CAG	CTT	GAG	GAT

	1875	1880	1885	1890	1895	1900	1905	1910	1915	1920						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	CCA	GAG	TTC	CAA	GCC	TCC	AAC	ATC	ATG	CAC	AGC	ATC	AAT	GGC	TAT	GTT
1. p25Dcod		1880		1890		1900		1910		1920						
( 16902 )	CCA	GAG	TTC	CAA	GCC	TCC	AAC	ATC	ATG	CAC	AGC	ATC	AAT	GGC	TAT	GTT>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	CCA	GAG	TTC	CAA	GCC	TCC	AAC	ATC	ATG	CAC	AGC	ATC	AAT	GGC	TAT	GTT

	1925	1930	1935	1940	1945	1950	1955	1960	1965							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	TTC	GAT	AGT	TTG	CAG	TTG	TCA	GTT	TGT	TTG	CAT	GAA	GTA	GCA	TAC	TGG
1. p25Dcod		1930		1940		1950		1960								
( 16902 )	TTt	GAT	AGT	TTG	CAG	TTG	TCA	GTT	TGT	TTG	CAT	GAg	GTg	GCA	TAC	TGG>
	AAv	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAv	AAv	AAA	AAA	AAA
pDJCcoding	TTC	GAT	AGT	TTG	CAG	TTG	TCA	GTT	TGT	TTG	CAT	GAA	GTA	GCA	TAC	TGG

Fig. 5G

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	1970	1975	1980	1985	1990	1995	2000	2005	2010	2015						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	TAC	ATT	CTA	AGC	ATT	GGA	GCA	CAG	ACT	GAC	TTC	CTT	TCT	GTC	TTC	TTC
1.p25Dcod	1970		1980		1990		2000		2010							
( 16902 )	TAC	ATT	CTA	AGC	ATT	GGA	GCA	CAG	ACT	GAC	TTC	CTT	TCT	GTC	TTC	TTC>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	TAC	ATT	CTA	AGC	ATT	GGA	GCA	CAG	ACT	GAC	TTC	CTT	TCT	GTC	TTC	TTC
	2020	2025	2030	2035	2040	2045	2050	2055	2060							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	TCT	GGA	TAT	ACC	TTC	AAA	CAC	AAA	ATG	GTC	TAT	GAA	GAC	ACA	CTC	ACC
1. p25Dcod	2020		2030		2040		2050		2060							
( 16902 )	TCT	GGA	TAT	ACC	TTC	AAA	CAC	AAA	ATG	GTC	TAT	GAA	GAC	ACA	CTC	ACC>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	TCT	GGA	TAT	ACC	TTC	AAA	CAC	AAA	ATG	GTC	TAT	GAA	GAC	ACA	CTC	ACC
	2065	2070	2075	2080	2085	2090	2095	2100	2105	2110						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	CTA	TTC	CCA	TTC	TCC	GGA	GAA	ACT	GTC	TTC	ATG	TCG	ATG	GAA	AAC	CCA
1. p25Dcod	2070		2080		2090		2100		2110							
( 16902 )	CTA	TTC	CCA	TTC	TCC	GGA	GAA	ACT	GTC	TTC	ATG	TCG	ATG	GAA	AAC	CCA>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	CTA	TTC	CCA	TTC	TCC	GGA	GAA	ACT	GTC	TTC	ATG	TCG	ATG	GAA	AAC	CCA
	2115	2120	2125	2130	2135	2140	2145	2150	2155	2160						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	GGA	CTA	TGG	ATT	CTG	GGG	TGC	CAC	AAC	TCA	GAC	TTT	CGG	AAC	AGA	GGC
1.p25Dcod	2120		2130		2140		2150		2160							
( 16902 )	GGT	CTA	TGG	ATT	CTG	GGG	TGC	CAC	AAC	TCA	GAC	TTT	CGG	AAC	AGA	GGC>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	GGA	CTA	TGG	ATT	CTG	GGG	TGC	CAC	AAC	TCA	GAC	TTT	CGG	AAC	AGA	GGC
	2165	2170	2175	2180	2185	2190	2195	2200	2205							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	ATG	ACC	GCC	TTA	CTG	AAA	GTT	TCC	AGT	TGT	GAC	AAG	AAC	ACT	GGA	GAT
1.p25Dcod	2170		2180		2190		2200									
( 16902 )	ATG	ACC	GCC	TTA	CTG	AAG	GTT	TCC	AGT	TGT	GAC	AAG	AAC	ACT	GGT	GAT>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	ATG	ACC	GCC	TTA	CTG	AAA	GTT	TCC	AGT	TGT	GAC	AAG	AAC	ACT	GGA	GAT
	2210	2215	2220	2225	2230	2235	2240	2245	2250	2255						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	TAT	TAC	GAG	GAC	AGT	TAT	GAA	GAT	ATT	TCA	GCA	TAC	TTG	CTG	AGT	AAA
1.p25Dc	2210		2220		2230		2240		2250							
( 16902 )	TAT	TAC	GAG	GAC	AGT	TAT	GAA	GAT	ATT	TCA	GCA	TAC	TTG	CTG	AGT	AAA>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	TAT	TAC	GAG	GAC	AGT	TAT	GAA	GAT	ATT	TCA	GCA	TAC	TTG	CTG	AGT	AAA

Fig. 5H

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	2260	2265	2270	2275	2280	2285	2290	2295	2300							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	AAC	AAT	GCC	ATT	GAA	CCA	AGA	AGC	TTC	TCC	CAG	AAC	CCA	CCA	GTC	TTG
1. p25Dcod	2260		2270		2280		2290		2300							
( 16902 )	AAC	AAT	GCC	ATT	GAA	CCA	AGA	AGC	TTC	TCC	CAG	AAC	CCA	CCA	GTC	TTG>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	AAC	AAT	GCC	ATT	GAA	CCA	AGA	AGC	TTC	TCC	CAG	AAC	CCA	CCA	GTC	TTG
	2305	2310	2315	2320	2325	2330	2335	2340	2345	2350						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	AAA	CGC	CAT	CAA	CGG	GAA	ATA	ACT	CGT	ACT	ACT	CTT	CAA	TCA	GAT	CAA
1. p25Dcod	2310		2320		2330		2340		2350							
( 16902 )	AAA	CGC	CAT	CAA	CGG	GAA	ATA	ACT	CGT	ACT	ACT	CTT	CAG	TCA	GAT	CAA>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	AAA	CGC	CAT	CAA	CGG	GAA	ATA	ACT	CGT	ACT	ACT	CTT	CAA	TCA	GAT	CAA
	2355	2360	2365	2370	2375	2380	2385	2390	2395	2400						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	GAG	GAA	ATT	GAC	TAT	GAT	ACC	ATA	TCA	GTT	GAA	ATG	AAG	AAG	GAA	
1. p25Dcod	2360		2370		2380		2390		2400							
( 16902 )	GAG	GAA	ATT	GAC	TAT	GAT	ACC	ATA	TCA	GTT	GAA	ATG	AAG	AAG	GAA>	
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	
pDJCcoding	GAG	GAA	ATT	GAC	TAT	GAT	ACC	ATA	TCA	GTT	GAA	ATG	AAG	AAG	GAA	
	2405	2410	2415	2420	2425	2430	2435	2440	2445							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	GAT	TTC	GAC	ATT	TAT	GAT	GAG	GAT	GAA	AAT	CAG	AGC	CCC	CGC	AGC	TTT
1. p25Dcod	2410		2420		2430		2440									
( 16902 )	GAT	TTC	GAC	ATT	TAT	GAT	GAG	GAT	GAA	AAT	CAG	AGC	CCC	CGC	AGC	TTT>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	GAT	TTC	GAC	ATT	TAT	GAT	GAG	GAT	GAA	AAT	CAG	AGC	CCC	CGC	AGC	TTT
	2450	2455	2460	2465	2470	2475	2480	2485	2490	2495						
pDJCcoding	CAA	AAG	AAA	ACA	CGA	CAC	TAT	TTT	ATT	GCT	GCA	GTG	GAG	AGG	CTC	TGG
1. p25Dc	2450		2460		2470		2480		2490							
( 16902 )	CAA	AAG	AAA	ACA	CGA	CAC	TAT	TTT	ATT	GCT	GCA	GTG	GAG	AGG	CTC	TGG>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	CAA	AAG	AAA	ACA	CGA	CAC	TAT	TTT	ATT	GCT	GCA	GTG	GAG	AGG	CTC	TGG
	2500	2505	2510	2515	2520	2525	2530	2535	2540							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	GAT	TAT	GGG	ATG	AGT	AGC	TCC	CCA	CAT	GTT	CTA	AGA	AAC	AGG	GCT	CAG
1. p25Dcod	2500		2510		2520		2530		2540							
( 16902 )	GAT	TAT	GGG	ATG	AGT	AGC	TCC	CCA	CAT	GTT	CTA	AGA	AAC	AGG	GCT	CAG>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	GAT	TAT	GGG	ATG	AGT	AGC	TCC	CCA	CAT	GTT	CTA	AGA	AAC	AGG	GCT	CAG

Fig. 51

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      2545   2550   2555   2560   2565   2570   2575   2580   2585   2590
      *     *     *     *     *     *     *     *     *     *
pDJCcoding  AGT GGC AGT GTC CCT CAG TTC AAG AAA GTA GTA TTC CAG GAA TTT ACC
      |
1. p25Dcod   2550           2560           2570           2580           2590
( 16902 )    AGT GGC AGT GTC CCT CAG TTC AAG AAA GTt GTt TTC CAG GAA TTT Act>
      AAA AAA AAA AAA AAA AAA AAA AAA AAy AAy AAA AAA AAA AAA AAy
pDJCcoding  AGT GGC AGT GTC CCT CAG TTC AAG AAA GTA GTA TTC CAG GAA TTT ACC

      2595   2600   2605   2610   2615   2620   2625   2630   2635   2640
      *     *     *     *     *     *     *     *     *     *
pDJCcoding  GAT GGC TCC TTT ACT CAA CCC TTA TAC CGT GGA GAA CTA AAT GAA CAT
      |
1.p25Dcod    2600           2610           2620           2630           2640
( 16902 )    GAT GGC TCC TTT ACT Cag CCC TTA TAC CGT GGA GAA CTA AAT GAA CAT>
      AAA AAA AAA AAA AAA AAy AAA AAA AAA AAA AAA AAA AAA AAA
pDJCcoding  GAT GGC TCC TTT ACT CAA CCC TTA TAC CGT GGA GAA CTA AAT GAA CAT

      2645   2650   2655   2660   2665   2670   2675   2680   2685
      *     *     *     *     *     *     *     *     *
pDJCcoding  TTG GGA CTC CTG GGG CCA TAT ATA AGA GCA GAA GTT GAA GAT AAT ATC
      |
1. p25Dcod    2650           2660           2670           2680
( 16902 )    TTG GGA CTC CTG GGG CCA TAT ATA AGA GCA GAA GTT GAA GAT AAT ATC>
      AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA
pDJCcoding  TTG GGA CTC CTG GGG CCA TAT ATA AGA GCA GAA GTT GAA GAT AAT ATC

      2690   2695   2700   2705   2710   2715   2720   2725   2730   2735
      *     *     *     *     *     *     *     *     *     *
pDJCcoding  ATG GTT ACC TTC AGA AAT CAG GCC TCT CGT CCC TAT TCC TTC TAT TCT
      |
1. p25Dc 2690           2700           2710           2720           2730
( 16902 )    ATG GTa ACt TTC AGA AAT CAG GCC TCT CGT CCC TAT TCC TTC TAT TCT>
      AAA AAy AAy AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA
pDJCcoding  ATG GTT ACC TTC AGA AAT CAG GCC TCT CGT CCC TAT TCC TTC TAT TCT

      2740   2745   2750   2755   2760   2765   2770   2775   2780
      *     *     *     *     *     *     *     *     *
pDJCcoding  TCC CTC ATA TCA TAT GAG GAA GAT CAG AGG CAA GGA GCA GAA CCT AGA
      |
1.p25 Dcod   2740           2750           2760           2770           2780
( 16902 )    agC Ctt Att Tct TAT GAG GAA GAT CAG AGG CAA GGA GCA GAA CCT AGA>
      vv^ AAy AAy AAy AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA
pDJCcoding  TCC CTC ATA TCA TAT GAG GAA GAT CAG AGG CAA GGA GCA GAA CCT AGA

      2785   2790   2795   2800   2805   2810   2815   2820   2825   2830
      *     *     *     *     *     *     *     *     *     *
pDJCcoding  AAA AAC TTT GTC AAG CCT AAT GAA ACC AAA ACT TAC TTT TGG AAA GTG
      |
1. p25 Dcod   2790           2800           2810           2820           2830
( 16902 )    AAA AAC TTT GTC AAG CCT AAT GAA ACC AAA ACT TAC TTT TGG AAA GTG>
      AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA
pDJCcoding  AAA AAC TTT GTC AAG CCT AAT GAA ACC AAA ACT TAC TTT TGG AAA GTG

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Fig. 5J

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	2835	2840	2845	2850	2855	2860	2865	2870	2875	2880
	*	*	*	*	*	*	*	*	*	*
pDJCcoding	CAA	CAT	CAT	ATG	GCA	CCC	ACT	AAA	GAT	GAG
1. p25Dcod		2840		2850		2860		2870		2880
( 16902 )	CAA	CAT	CAT	ATG	GCA	CCC	ACT	AAA	GAT	GAG
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	CAA	CAT	CAT	ATG	GCA	CCC	ACT	AAA	GAT	GAG
	2885	2890	2895	2900	2905	2910	2915	2920	2925	
	*	*	*	*	*	*	*	*	*	
pDJCcoding	GCT	TAT	TTC	TCC	GAT	GTC	GAC	CTG	GAA	AAA
( 16902 )	GCT	TAT	TTC	TCC	GAT	GTC	GAC	CTG	GAA	AAA
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	GCT	TAT	TTC	TCC	GAT	GTC	GAC	CTG	GAA	AAA
	2930	2935	2940	2945	2950	2955	2960	2965	2970	2975
	*	*	*	*	*	*	*	*	*	*
pDJCcoding	ATT	GGA	CCC	CTT	CTG	GTC	TGC	CAC	ACC	AAC
1.p25Dc	2930		2940		2950		2960		2970	
( 16902 )	ATT	GGA	CCC	CTT	CTG	GTC	TGC	CAC	ACC	AAC
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	ATT	GGA	CCC	CTT	CTG	GTC	TGC	CAC	ACC	AAC
	2980	2985	2990	2995	3000	3005	3010	3015	3020	
	*	*	*	*	*	*	*	*	*	
pDJCcoding	GGG	AGA	CAA	GTG	ACA	GTA	CAG	GAA	TTT	GCT
1. p25Dcod	2980		2990		3000		3010		3020	
( 16902 )	GGG	AGA	CAA	GTG	ACA	GTA	CAG	GAA	TTT	GCT
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	GGG	AGA	CAA	GTG	ACA	GTA	CAG	GAA	TTT	GCT
	3025	3030	3035	3040	3045	3050	3055	3060	3065	3070
	*	*	*	*	*	*	*	*	*	*
pDJCcoding	GAT	GAG	ACC	AAA	AGC	TGG	TAC	TTC	ACT	GAA
1. p25Dcod		3030		3040		3050		3060		3070
( 16902 )	GAT	GAG	ACC	AAA	AGC	TGG	TAC	TTC	ACT	GAA
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	GAT	GAG	ACC	AAA	AGC	TGG	TAC	TTC	ACT	GAA
	3075	3080	3085	3090	3095	3100	3105	3110	3115	3120
	*	*	*	*	*	*	*	*	*	*
pDJCcoding	AGG	GCT	CCC	TGC	AAT	ATC	CAG	ATG	GAA	GAT
1.p25Dcod		3080		3090		3100		3110		3120
( 16902 )	AGG	GCT	CCC	TGC	AAT	ATC	CAG	ATG	GAA	GAT
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	AGG	GCT	CCC	TGC	AAT	ATC	CAG	ATG	GAA	GAT

Fig. 5K

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	3125	3130	3135	3140	3145	3150	3155	3160	3165							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	TAT	CGC	TTC	CAT	GCA	ATC	AAT	GGC	TAC	ATA	ATG	GAT	ACA	CTA	CCT	GGC
1. p25Dcod			3130		3140		3150		3160							
( 16902 )	TAT	CGC	TTC	CAT	GCA	ATC	AAT	GGC	TAC	ATA	ATG	GAT	ACA	CTA	CCT	GGC>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	TAT	CGC	TTC	CAT	GCA	ATC	AAT	GGC	TAC	ATA	ATG	GAT	ACA	CTA	CCT	GGC

	3170	3175	3180	3185	3190	3195	3200	3205	3210	3215						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	TTA	GTA	ATG	GCT	CAG	GAT	CAA	AGG	ATT	CGA	TGG	TAT	CTG	CTC	AGC	ATG
1.p25Dcod	3170		3180		3190		3200		3210							
( 16902 )	TTA	GTA	ATG	GCT	CAG	GAT	CAA	AGG	ATT	CGA	TGG	TAT	CTG	CTC	AGC	ATG>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	TTA	GTA	ATG	GCT	CAG	GAT	CAA	AGG	ATT	CGA	TGG	TAT	CTG	CTC	AGC	ATG

	3220	3225	3230	3235	3240	3245	3250	3255	3260							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	GGC	AGC	AAT	GAA	AAC	ATC	CAT	TCT	ATT	CAT	TTC	TCC	GGA	CAT	GTG	TTC
1. p25Dcod	3220		3230		3240		3250		3260							
( 16902 )	GGC	AGC	AAT	GAA	AAC	ATC	CAT	TCT	ATT	CAT	TTC	agt	GGA	CAT	GTG	TTC>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	vvv	AAA	AAA	AAA	AAA
pDJCcoding	GGC	AGC	AAT	GAA	AAC	ATC	CAT	TCT	ATT	CAT	TTC	TCC	GGA	CAT	GTG	TTC

	3265	3270	3275	3280	3285	3290	3295	3300	3305	3310						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	ACT	GTA	CGA	AAA	AAA	GAG	GAG	TAT	AAA	ATG	GCA	CTG	TAC	AAT	CTC	TAT
1.p25Dcod		3270		3280		3290		3300		3310						
( 16902 )	ACT	GTA	CGA	AAA	AAA	GAG	GAG	TAT	AAA	ATG	GCA	CTG	TAC	AAT	CTC	TAT>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	ACT	GTA	CGA	AAA	AAA	GAG	GAG	TAT	AAA	ATG	GCA	CTG	TAC	AAT	CTC	TAT

	3315	3320	3325	3330	3335	3340	3345	3350	3355	3360						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	CCC	GGA	GTT	TTC	GAG	ACA	GTG	GAA	ATG	TTA	CCA	TCC	AAA	GCT	GGA	ATT
1.p25Dcod		3320		3330		3340		3350		3360						
( 16902 )	CCa	GGt	GTT	TTt	GAG	ACA	GTG	GAA	ATG	TTA	CCA	TCC	AAA	GCT	GGA	ATT>
	AAv	AAv	AAA	AAv	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	CCC	GGA	GTT	TTC	GAG	ACA	GTG	GAA	ATG	TTA	CCA	TCC	AAA	GCT	GGA	ATT

	3365	3370	3375	3380	3385	3390	3395	3400	3405							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	TGG	CGG	GTG	GAA	TGC	CTT	ATT	GGC	GAG	CAT	CTA	CAT	GCT	GGG	ATG	AGC
1.p25Dcod		3370		3380		3390		3400								
( 16902 )	TGG	CGG	GTG	GAA	TGC	CTT	ATT	GGC	GAG	CAT	CTA	CAT	GCT	GGG	ATG	AGC>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	TGG	CGG	GTG	GAA	TGC	CTT	ATT	GGC	GAG	CAT	CTA	CAT	GCT	GGG	ATG	AGC

Fig. 5L

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      3410   3415   3420   3425   3430   3435   3440   3445   3450   3455
      *     *     *     *     *     *     *     *     *     *
pDJCcoding ACA CTT TTT CTG GTG TAC TCC AAT AAG TGT CAG ACT CCC CTG GGA ATG
      |           |           |           |           |
1.p25Dcod 3410           3420           3430           3440           3450
( 16902 ) ACA CTT TTT CTG GTG TAC agC AAT AAG TGT CAG ACT CCC CTG GGA ATG>
      |           |           |           |           |
pDJCcoding ACA CTT TTT CTG GTG TAC TCC AAT AAG TGT CAG ACT CCC CTG GGA ATG

      3460   3465   3470   3475   3480   3485   3490   3495   3500
      *     *     *     *     *     *     *     *     *
pDJCcoding GCT TCT GGA CAC ATT AGA GAT TTT CAG ATT ACA GCT TCA GGA CAA TAT
      |           |           |           |           |
1. p25Dcod 3460           3470           3480           3490           3500
( 16902 ) GCT TCT GGA CAC ATT AGA GAT TTT CAG ATT ACA GCT TCA GGA CAA TAT>
      |           |           |           |           |
pDJCcoding GCT TCT GGA CAC ATT AGA GAT TTT CAG ATT ACA GCT TCA GGA CAA TAT

      3505   3510   3515   3520   3525   3530   3535   3540   3545   3550
      *     *     *     *     *     *     *     *     *     *
pDJCcoding GGA CAG TGG GCC CCA AAG CTG GCC AGA CTT CAT TAT TCC GGA TCA ATC
      |           |           |           |           |
1.p25Dcod 3510           3520           3530           3540           3550
( 16902 ) GGA CAG TGG GCC CCA AAG CTG GCC AGA CTT CAT TAT TCC GGA TCA ATC>
      |           |           |           |           |
pDJCcoding GGA CAG TGG GCC CCA AAG CTG GCC AGA CTT CAT TAT TCC GGA TCA ATC

      3555   3560   3565   3570   3575   3580   3585   3590   3595   3600
      *     *     *     *     *     *     *     *     *     *
pDJCcoding AAT GCC TGG AGC ACC AAG GAG CCC TTT TCT TGG ATC AAA GTT GAC CTG
      |           |           |           |           |
1. p25Dcod 3560           3570           3580           3590           3600
( 16902 ) AAT GCC TGG AGC ACC AAG GAG CCC TTT TCT TGG ATC AAg GTg Gat CTG>
      |           |           |           |           |
pDJCcoding AAT GCC TGG AGC ACC AAG GAG CCC TTT TCT TGG ATC AAA GTT GAC CTG

      3605   3610   3615   3620   3625   3630   3635   3640   3645
      *     *     *     *     *     *     *     *     *
pDJCcoding TTG GCA CCA ATG ATT ATT CAC GGC ATC AAG ACC CAG GGT GCC CGT CAG
      |           |           |           |           |
1.P25Dcod 3610           3620           3630           3640
( 16902 ) TTG GCA CCA ATG ATT ATT CAC GGC ATC AAG ACC CAG GGT GCC CGT CAG>
      |           |           |           |           |
pDJCcoding TTG GCA CCA ATG ATT ATT CAC GGC ATC AAG ACC CAG GGT GCC CGT CAG

      3650   3655   3660   3665   3670   3675   3680   3685   3690   3695
      *     *     *     *     *     *     *     *     *     *
pDJCcoding AAG TTC TCC AGC CTC TAC ATC TCT CAA TTT ATC ATC ATG TAT AGT CTC
      |           |           |           |           |
1. p25Dc 3650           3660           3670           3680           3690
( 16902 ) AAG TTC TCC AGC CTC TAC ATC TCT CAg TTT ATC ATC ATG TAT AGT CTt>
      |           |           |           |           |
pDJCcoding AAG TTC TCC AGC CTC TAC ATC TCT CAA TTT ATC ATC ATG TAT AGT CTC

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Fig. 5M

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      3700  3705  3710  3715  3720  3725  3730  3735  3740
      *    *    *    *    *    *    *    *    *
pDJCcoding  GAT GGG AAG AAG TGG CAG ACT TAT CGA GGA AAT TCC ACT GGA ACC CTC
              |      |      |      |      |
1. p25Dcod  3700      3710      3720      3730      3740
( 16902 )   GAT GGG AAG AAG TGG CAG ACT TAT CGA GGA AAT TCC ACT GGA ACC tTa>
              |      |      |      |      |
pDJCcoding  GAT GGG AAG AAG TGG CAG ACT TAT CGA GGA AAT TCC ACT GGA ACC CTC

      3745  3750  3755  3760  3765  3770  3775  3780  3785  3790
      *    *    *    *    *    *    *    *    *
pDJCcoding  ATG GTC TTC TTT GGC AAT GTG GAT TCA TCT GGG ATA AAA CAC AAT ATT
              |      |      |      |      |
1. p25Dcod  3750      3760      3770      3780      3790
( 16902 )   ATG GTC TTC TTT GGC AAT GTG GAT TCA TCT GGG ATA AAA CAC AAT ATT>
              |      |      |      |      |
pDJCcoding  ATG GTC TTC TTT GGC AAT GTG GAT TCA TCT GGG ATA AAA CAC AAT ATT

      3795  3800  3805  3810  3815  3820  3825  3830  3835  3840
      *    *    *    *    *    *    *    *    *
pDJCcoding  TTC AAC CCT CCA ATT ATT GCT CGA TAC ATC CGT TTG CAC CCA ACT CAT
              |      |      |      |      |
1. p25Dcod  3800      3810      3820      3830      3840
( 16902 )   TTC AAC CCT CCA ATT ATT GCT CGA TAC ATC CGT TTG CAC CCA ACT CAT>
              |      |      |      |      |
pDJCcoding  TTC AAC CCT CCA ATT ATT GCT CGA TAC ATC CGT TTG CAC CCA ACT CAT

      3845  3850  3855  3860  3865  3870  3875  3880  3885
      *    *    *    *    *    *    *    *
pDJCcoding  TAT AGC ATT CGC AGC ACT CTT CGC ATG GAG TTG ATG GGC TGT GAT TTA
              |      |      |      |      |
1. p25Dcod  3850      3860      3870      3880
( 16902 )   TAT AGC ATT CGC AGC ACT CTT CGC ATG GAG TTG ATG GGC TGT GAT TTA>
              |      |      |      |      |
pDJCcoding  TAT AGC ATT CGC AGC ACT CTT CGC ATG GAG TTG ATG GGC TGT GAT TTA

      3890  3895  3900  3905  3910  3915  3920  3925  3930  3935
      *    *    *    *    *    *    *    *
pDJCcoding  AAT AGT TGC AGC ATG CCA TTG GGA ATG GAG AGT AAA GCA ATA TCA GAT
              |      |      |      |      |
1. p25Dc 3890      3900      3910      3920      3930
( 16902 )   AAT AGT TGC AGC ATG CCA TTG GGA ATG GAG AGT AAA GCA ATA TCA GAT>
              |      |      |      |      |
pDJCcoding  AAT AGT TGC AGC ATG CCA TTG GGA ATG GAG AGT AAA GCA ATA TCA GAT

      3940  3945  3950  3955  3960  3965  3970  3975  3980
      *    *    *    *    *    *    *    *
pDJCcoding  GCA CAG ATT ACT GCT TCA TCC TAC TTT ACC AAT ATG TTT GCC ACC TGG
              |      |      |      |      |
1. p25Dcod  3940      3950      3960      3970      3980
( 16902 )   GCA CAG ATT ACT GCT TCA TCC TAC TTT ACC AAT ATG TTT GCC ACC TGG>
              |      |      |      |      |
pDJCcoding  GCA CAG ATT ACT GCT TCA TCC TAC TTT ACC AAT ATG TTT GCC ACC TGG

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Fig. 5N

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	3985	3990	3995	4000	4005	4010	4015	4020	4025	4030						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	TCT	CCT	TCA	AAA	GCT	CGA	CTA	CAC	CTA	CAA	GGG	AGG	AGT	AAT	GCC	TGG
1. P25Dcod		3990		4000		4010		4020		4030						
( 16902 )	TCT	CCT	TCA	AAA	GCT	CGA	CTt	CAC	CTc	CAA	GGG	AGG	AGT	AAT	GCC	TGG>
	AAA	AAA	AAA	AAA	AAA	AAA	AAv	AAA	AAv	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	TCT	CCT	TCA	AAA	GCT	CGA	CTA	CAC	CTA	CAA	GGG	AGG	AGT	AAT	GCC	TGG

	4035	4040	4045	4050	4055	4060	4065	4070	4075	4080						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	AGA	CCT	CAA	GTT	AAC	AAT	CCA	AAA	GAG	TGG	CTG	CAA	GTG	GAC	TTC	CAG
1. p25Dcod		4040		4050		4060		4070		4080						
( 16902 )	AGA	CCT	CAG	GTg	AAt	AAT	CCA	AAA	GAG	TGG	CTG	CAA	GTG	GAC	TTC	CAG>
	AAA	AAA	AAv	AAv	AAv	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	AGA	CCT	CAA	GTT	AAC	AAT	CCA	AAA	GAG	TGG	CTG	CAA	GTG	GAC	TTC	CAG

	4085	4090	4095	4100	4105	4110	4115	4120	4125							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	AAG	ACA	ATG	AAA	GTC	ACA	GGA	GTA	ACT	ACT	CAG	GGA	GTA	AAA	TCT	CTG
1. p25Dcod		4090		4100		4110		4120								
( 16902 )	AAG	ACA	ATG	AAA	GTC	ACA	GGA	GTA	ACT	ACT	CAG	GGA	GTA	AAA	TCT	CTG
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	AAG	ACA	ATG	AAA	GTC	ACA	GGA	GTA	ACT	ACT	CAG	GGA	GTA	AAA	TCT	CTG

	4130	4135	4140	4145	4150	4155	4160	4165	4170	4175						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	CTT	ACC	TCT	ATG	TAC	GTG	AAG	GAG	TTC	CTC	ATA	TCG	TCG	TCG	CAA	GAT
1. p25Dc	4130		4140		4150		4160		4170							
( 16902 )	CTT	ACC	agc	ATG	TAt	GTG	AAG	GAG	TTC	CTC	ATc	TCc	agc	agt	CAA	GAT>
	AAA	AAA	vvv	AAA	AAv	AAA	AAA	AAA	AAA	AAA	AAv	AAv	vvv	vvv	AAA	AAA
pDJCcoding	CTT	ACC	TCT	ATG	TAC	GTG	AAG	GAG	TTC	CTC	ATA	TCG	TCG	TCG	CAA	GAT

	4180	4185	4190	4195	4200	4205	4210	4215	4220							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	GGC	CAT	CAG	TGG	ACT	CTC	TTT	TTT	CAA	AAT	GGC	AAA	GTA	AAA	GTT	TTC
1. p25Dcod	4180		4190		4200		4210		4220							
( 16902 )	GGC	CAT	CAG	TGG	ACT	CTC	TTT	TTT	CAG	AAT	GGC	AAA	GTA	AAg	GTT	TTt>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAv	AAA	AAA	AAA	AAA	AAv	AAA	AAv
pDJCcoding	GGC	CAT	CAG	TGG	ACT	CTC	TTT	TTT	CAA	AAT	GGC	AAA	GTA	AAA	GTT	TTC

	4225	4230	4235	4240	4245	4250	4255	4260	4265	4270						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	CAG	GGA	AAT	CAA	GAC	TCC	TTC	ACA	CCT	GTC	GTG	AAC	TCT	CTA	GAC	CCA
1. p25Dcod		4230		4240		4250		4260		4270						
( 16902 )	CAG	GGA	AAT	CAA	GAC	TCC	TTC	ACA	CCT	GTg	GTG	AAC	TCT	CTA	GAC	CCA>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAv	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	CAG	GGA	AAT	CAA	GAC	TCC	TTC	ACA	CCT	GTC	GTG	AAC	TCT	CTA	GAC	CCA

Fig. 50

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	4275	4280	4285	4290	4295	4300	4305	4310	4315	4320						
	*	*	*	*	*	*	*	*	*	*						
pDJCcoding	CCG	TTA	CTC	ACT	CGC	TAC	CTT	CGA	ATT	CAC	CCC	CAG	AGT	TGG	GTG	CAC
1. p25Dcod		4280		4290		4300		4310		4320						
( 16902 )	CCG	TTA	CTg	ACT	CGC	TAC	CTT	CGA	ATT	CAC	CCC	CAG	AGT	TGG	GTG	CAC>
	AAA	AAA	AAv	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	CCG	TTA	CTC	ACT	CGC	TAC	CTT	CGA	ATT	CAC	CCC	CAG	AGT	TGG	GTG	CAC
	4325	4330	4335	4340	4345	4350	4355	4360	4365							
	*	*	*	*	*	*	*	*	*							
pDJCcoding	CAG	ATT	GCC	CTG	AGG	ATG	GAG	GTT	CTG	GGC	TGC	GAG	GCA	CAG	GAC	CTC
1. p25Dcod		4330		4340		4350		4360								
( 16902 )	CAG	ATT	GCC	CTG	AGG	ATG	GAG	GTT	CTG	GGC	TGC	GAG	GCA	CAG	GAC	CTC>
	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
pDJCcoding	CAG	ATT	GCC	CTG	AGG	ATG	GAG	GTT	CTG	GGC	TGC	GAG	GCA	CAG	GAC	CTC
	4370															
	*															
pDJCcoding	TAC	TGA														
1. p25Dc	4370															
( 16902 )	TAC	TGA>														
	AAA	AAA														
pDJCcoding	TAC	TGA														

Fig. 5P

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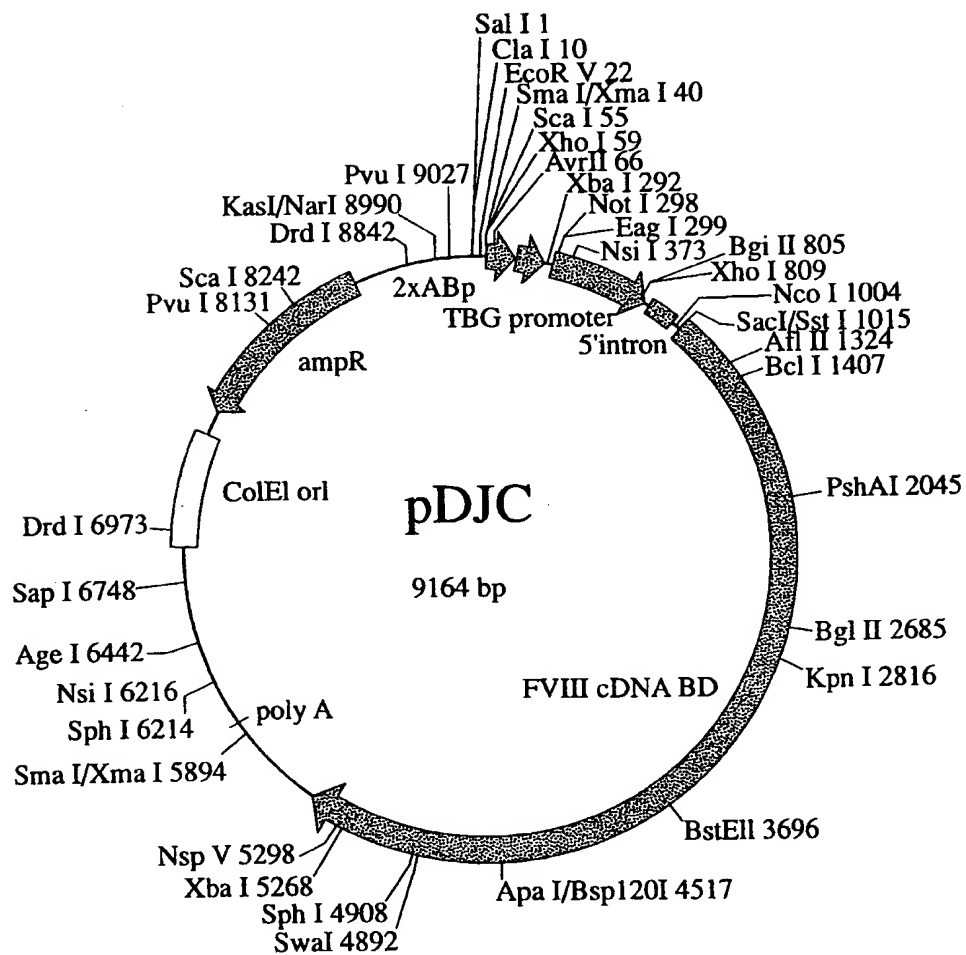


Fig. 6

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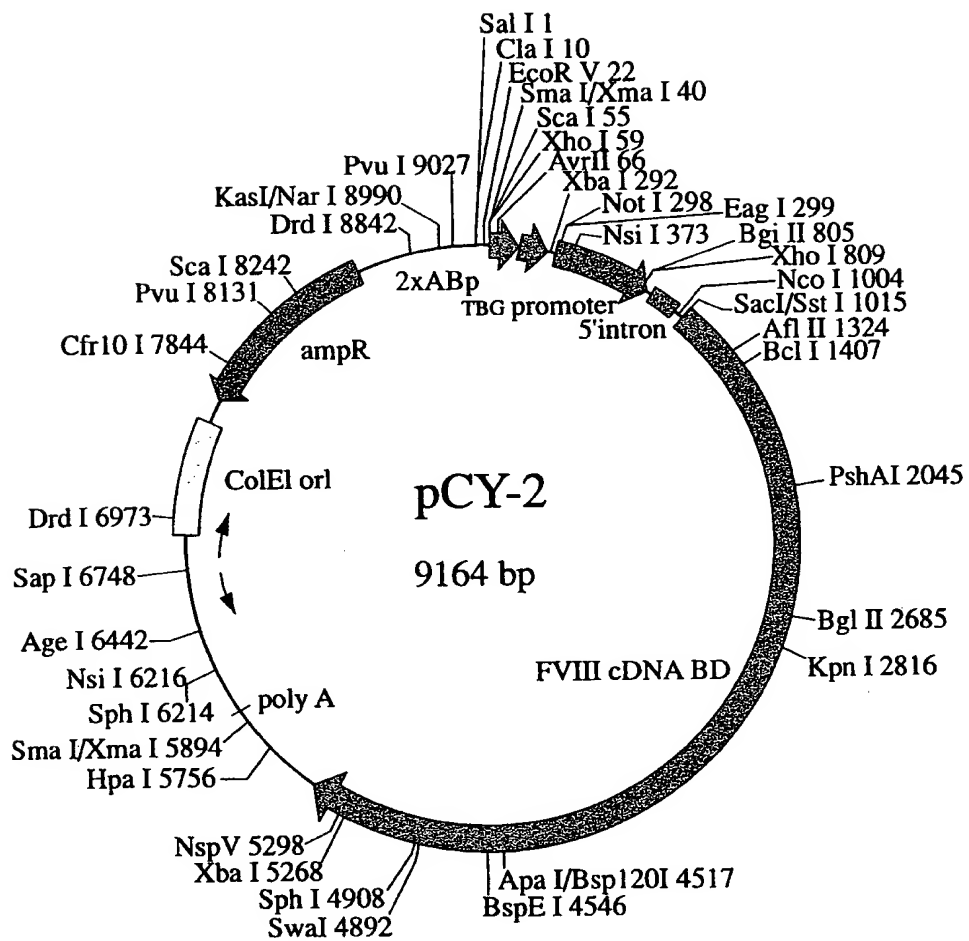
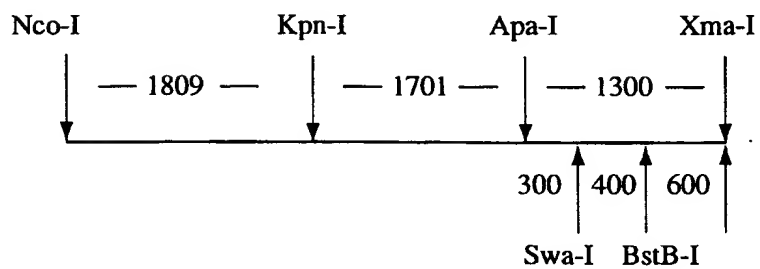


Fig. 7

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**Human Factor VIII cDNA Synthetic Approach****Fig. 8**

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Generate PCR Fragments      Clone into pBluescript and sequence      Clone into Factor expression Plasmid

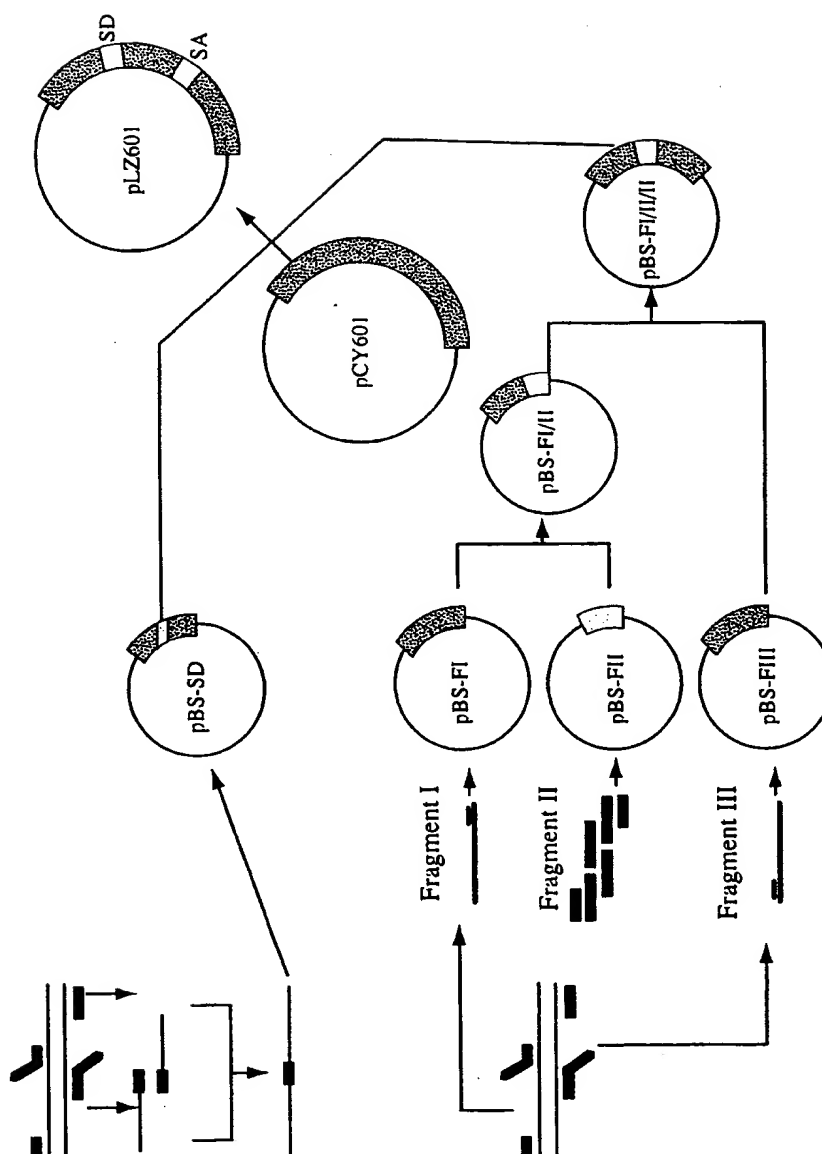


Fig. 9



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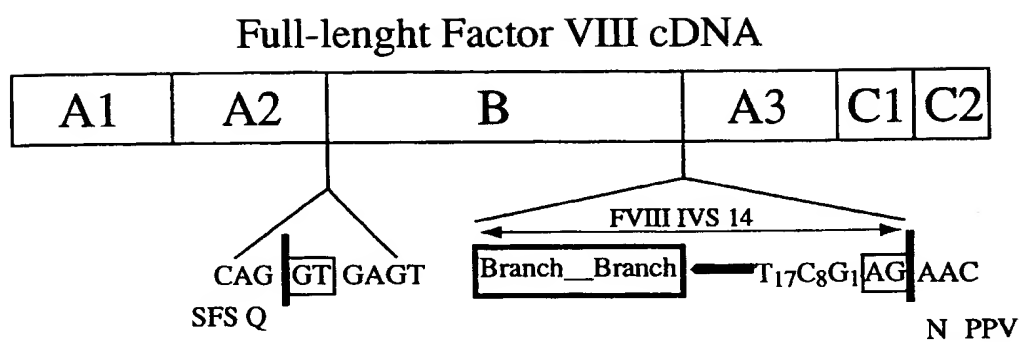


Fig. 10

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### Factor VII Full Length cDNA (Exon-Intro Boundary)

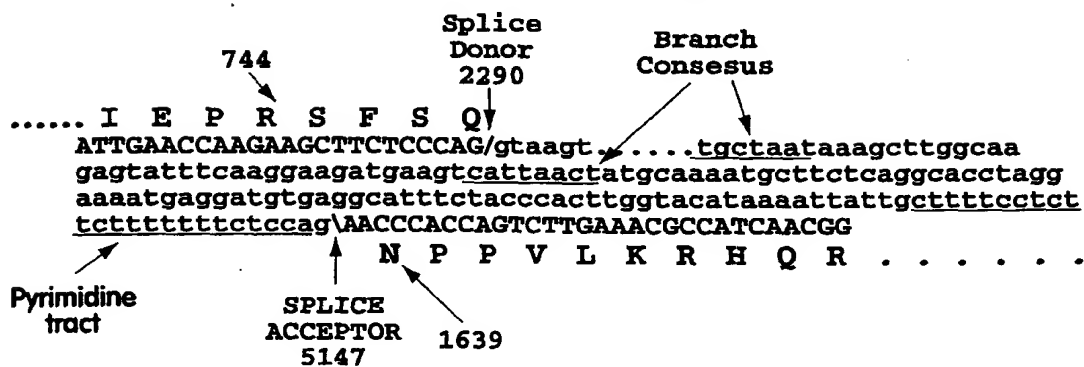


Fig. 11

### Human Factor VIII cDNA: Intron Engineering Considerations

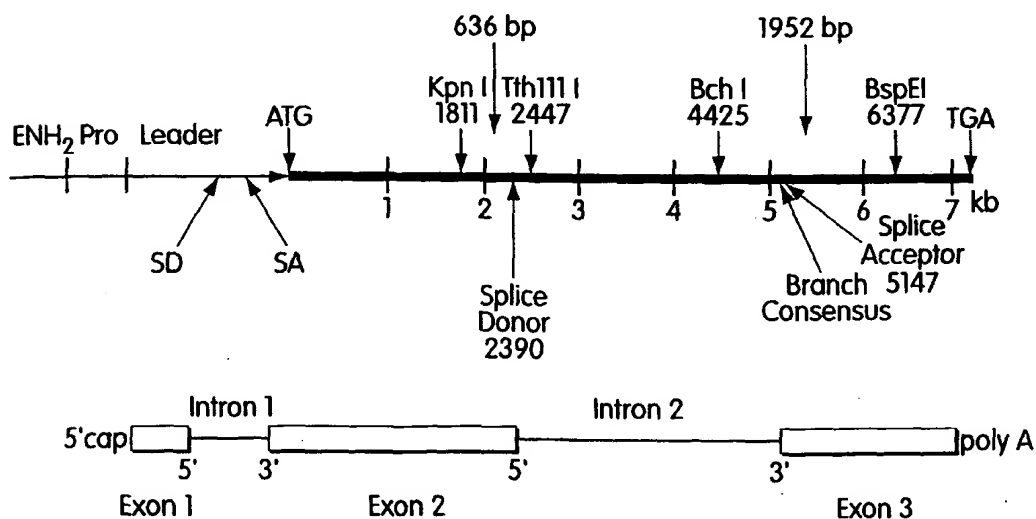


Fig. 12

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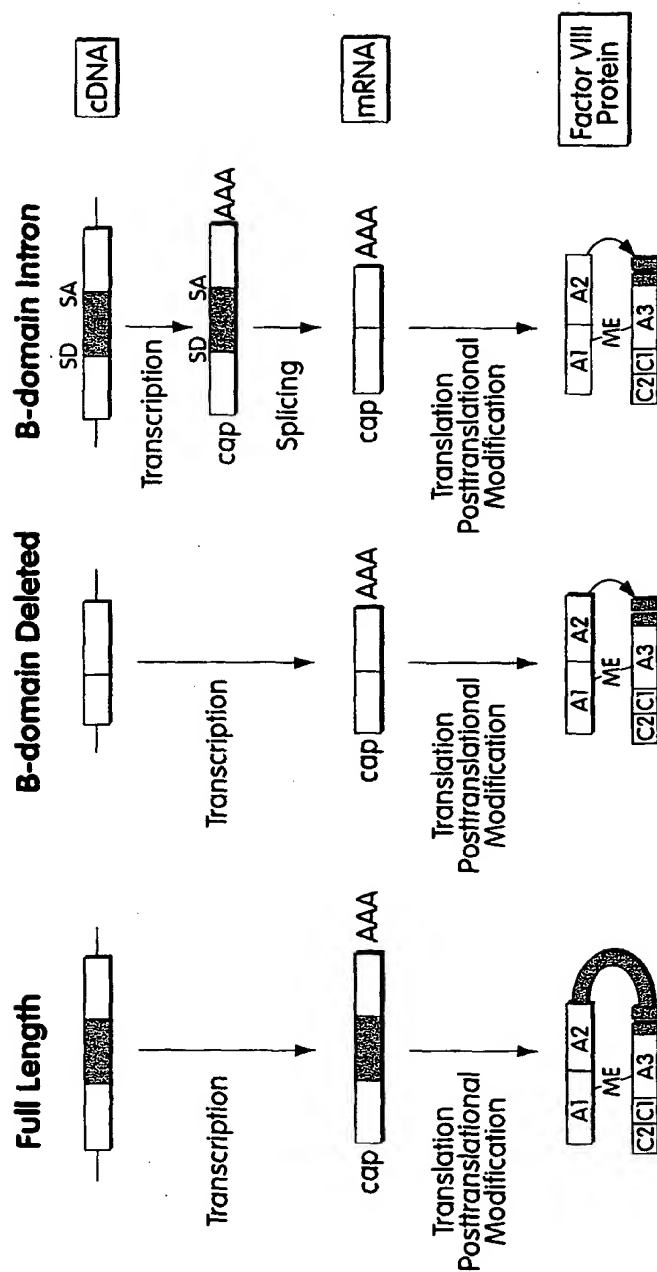


Fig. 13

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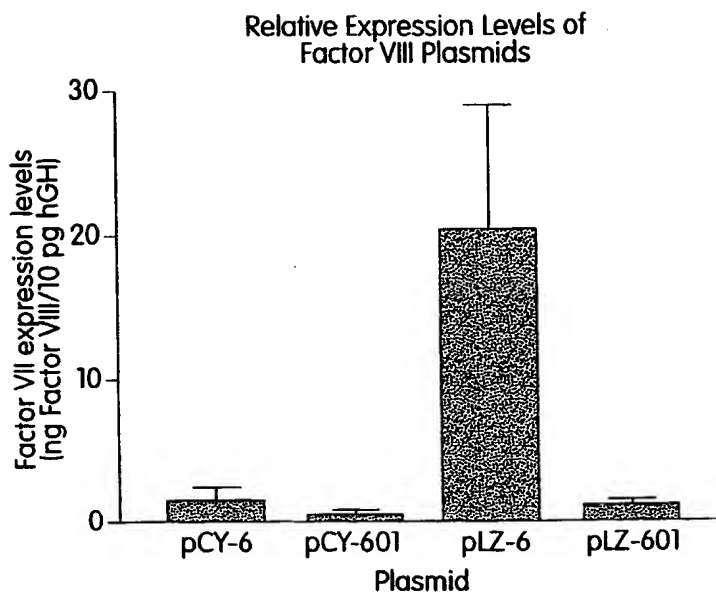


Fig. 14

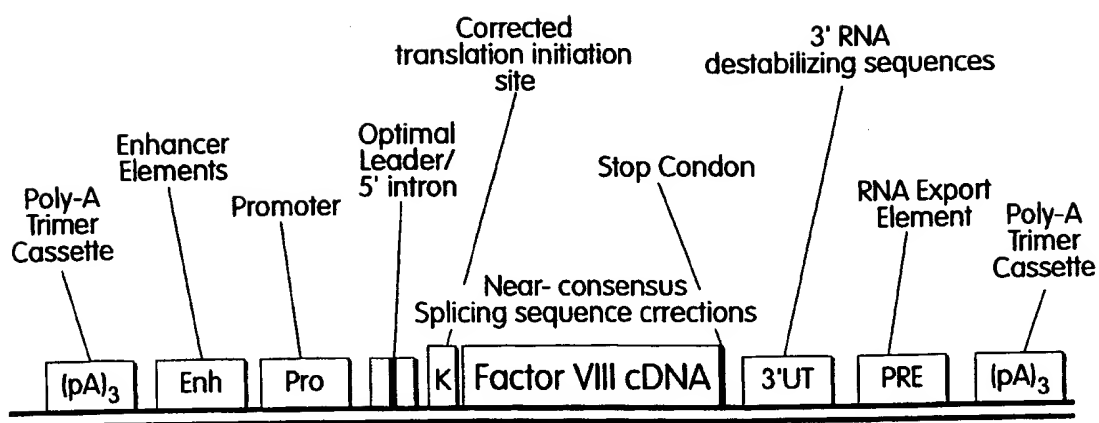


Fig. 15

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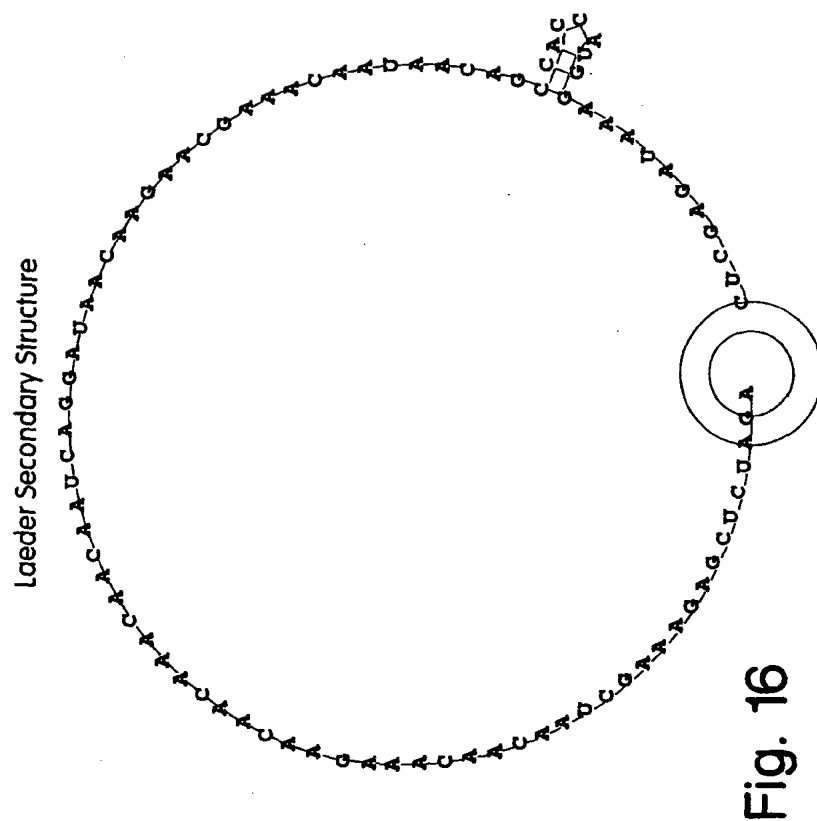
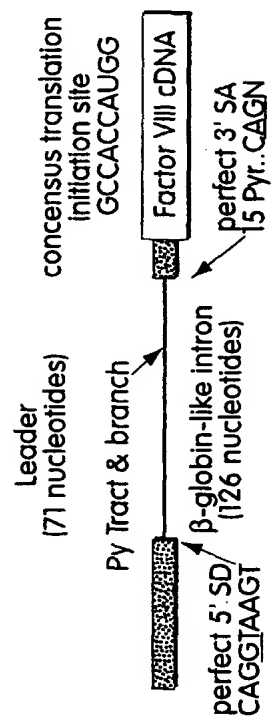


Fig. 16



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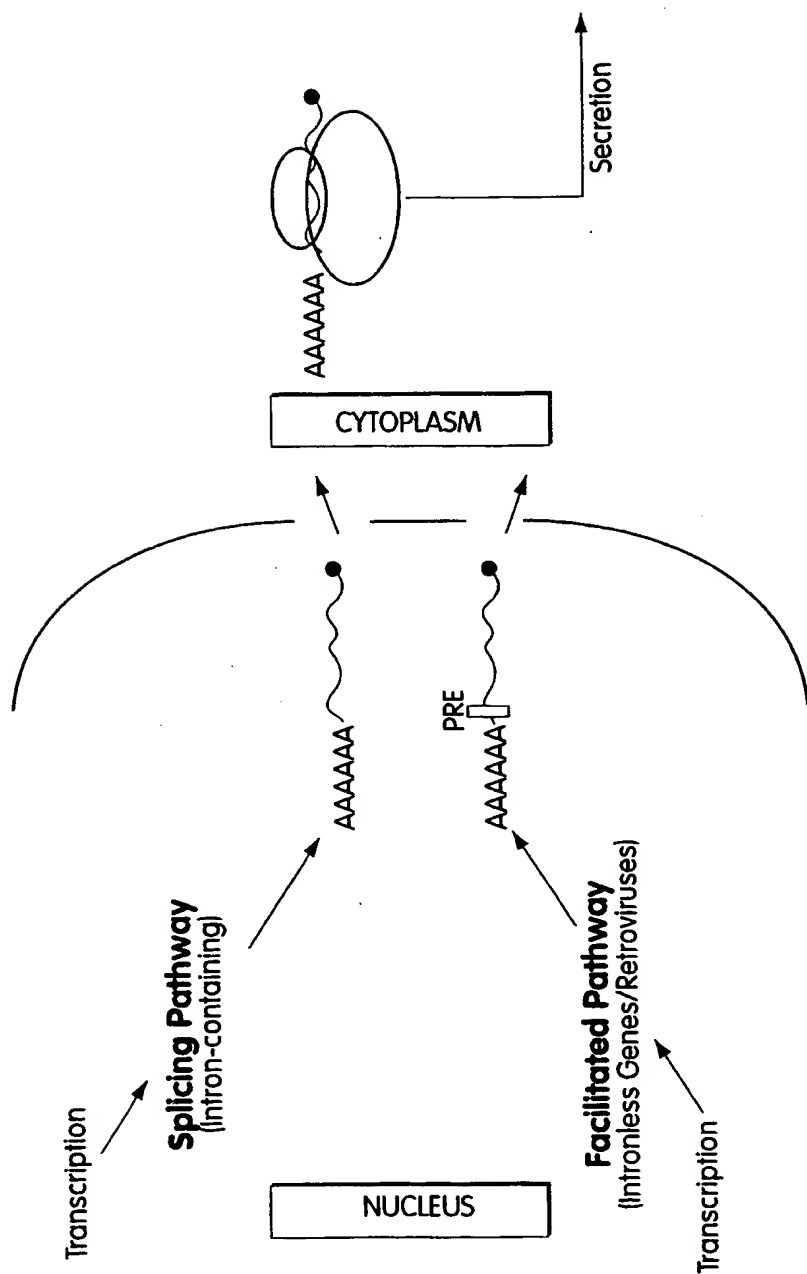


Fig. 17

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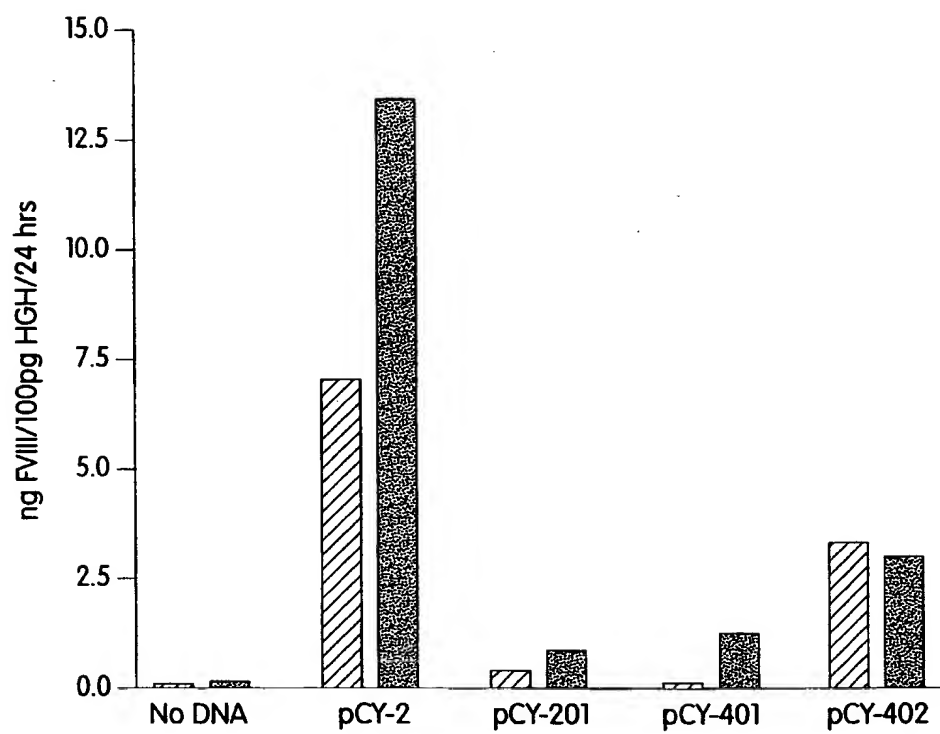


Fig. 18

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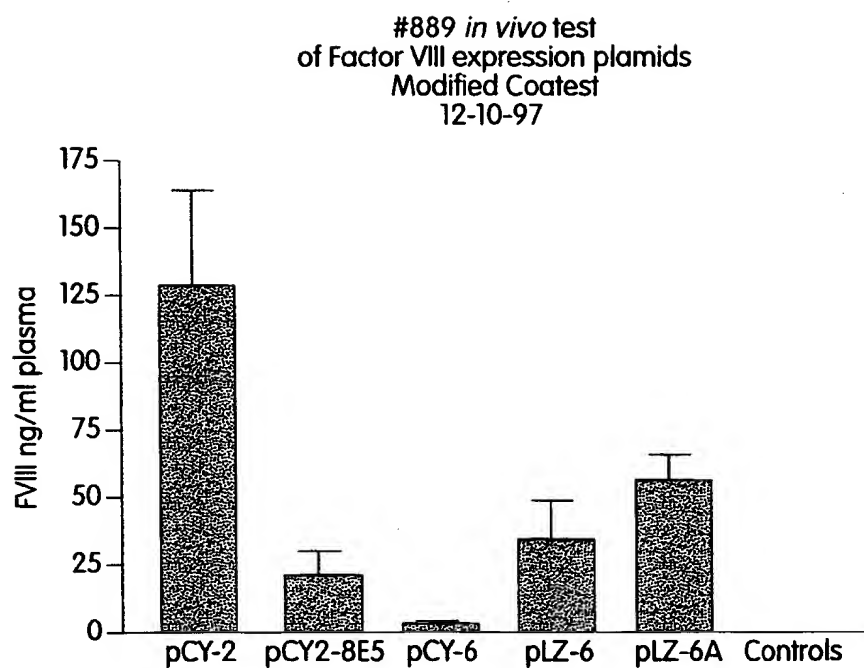


Fig. 19



## A Potent Tissue-specific Enhancer Made of Clustered Liver-specific Elements from the Human Alpha-1 Microglobulin/Bikunin Gene\*

5' AGGTTAATTT TTAAAAAGCA GTCAAAGTC CAAGTGGCCC TTGCGAGCAT  
HNF-1 HNF-4  
TTACTCTCTC TGTTTGCTCT GGTTAATAAT CTCAGGAGC A CAAACATTCC  
HNF-3 HNF-1 HNF-3 3'

From: P. Rouet...J.P. Sailer, (1992) J.Biol. Chem 267 No.29,pp. 20765-20773

## The Immune Response Corporation

Fig. 20

### A 218-bp Fragment of the Human Thyroxine-Binding Globulin (TBG) Gene Contains Full Promoter Activity

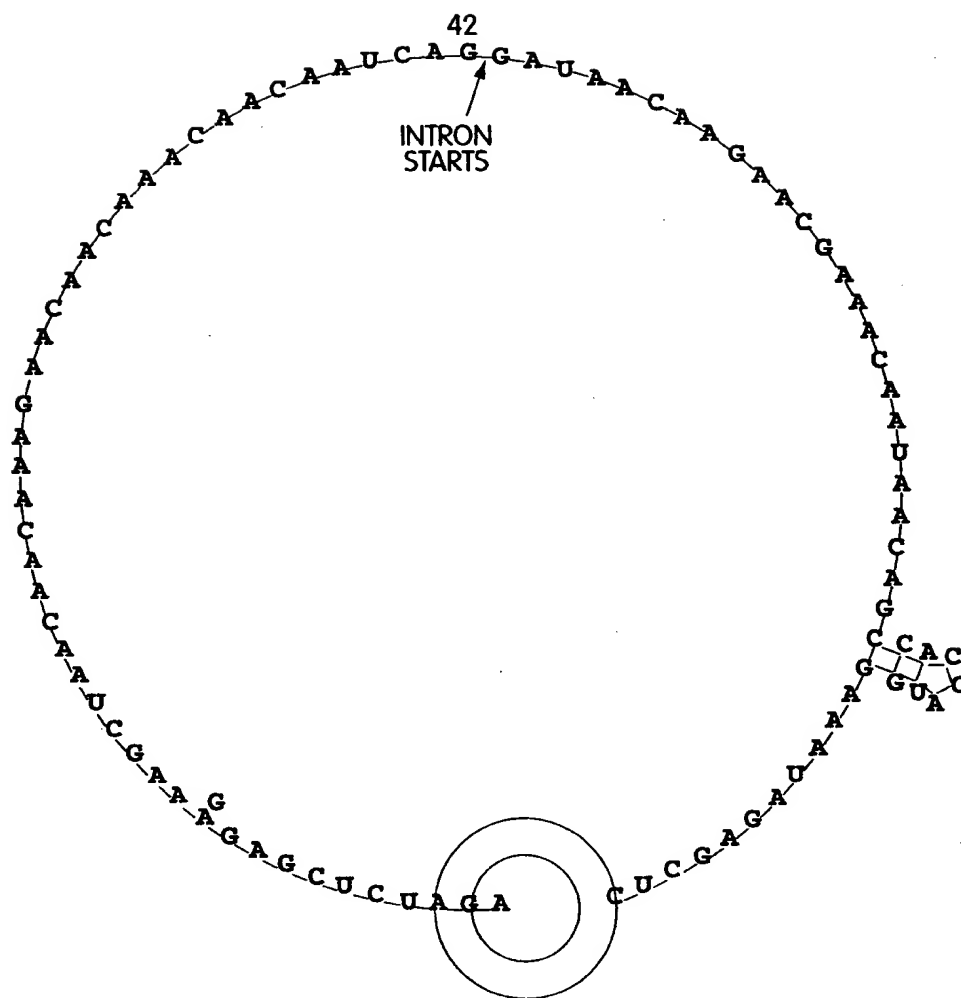
CTTTCTCT TTTCTTTTAC ATGAA GGGTCTGGCAGCCAAA GCAAT CACT CAAAGTTCAAA  
 AP-1 HNF-3  
 CTTTCTCT TTTTGGCTTTGT TCCTCTTGG CTTGGTTTGT TACATCAG CTTTGAATAAT  
 HNF-3  
 ACCATCCCCAG GGTAAATGCT GGG GTTAATTATAAC TAAGAGTGCTCTAG TTTT GCAAT  
 ACAGGACATGC TATAA AAATGGAACAGATGTTGCTTTCTGAGAGATA  
 5 3'

From: Y. Hayashi.....S. Refetoff.(1993), *Molec. Endo.* 7 NO. 8, pp. 1049-1060

The Immune Response Corporation

**Fig. 21**

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**Fig. 22**

[illegible]

**Fig. 23**

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## Endothelium-Specific Promoters and Enhancers



Promoter Name	Characteristics	Reference
1) Endothelin-1	204 bp; has enhancer seq, TATAA box, TS mapped	Lee et. al. <i>JBC</i> 265 No. 18, 1990. →
2) Flt-1 (fms-like tyrosine kinase)	~1 kb, no enhancer, TATAA box TS mapped	Morishita et. al. <i>JBC</i> 270 No. 46, 1995
3) Nitric Oxide Synthase	~1 kb, GATAA box, TS mapped	Zhang et. al. <i>JBC</i> 270 NO 25, 1995
<b>Enhancers</b>		
1) c-Fos SRE	60 nt's, non-tissue specific, active in resting or dividing cells	Treisman, <i>Cell</i> 46, 1986 →
2) hTF/mTie-2	Hybrid design, 72 nt's, all endo's	1998

Fig. 24

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### Human Factor VIII In Vivo Expression Viral vs Tissue-specific Promoter

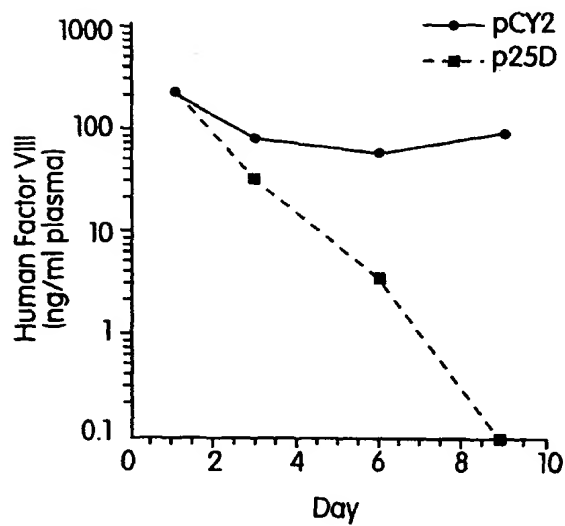


Fig. 25

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## SEQUENCE LISTING

(1) GENERAL INFORMATION:

5 (i) APPLICANT:  
(A) NAME: THE IMMUNE RESPONSE CORPORATION  
(B) STREET: 5935 DARWIN COURT  
(C) CITY: CARLSBAD  
10 (D) STATE: CALIFORNIA  
(E) COUNTRY: US  
(F) POSTAL CODE (ZIP): 92008  
(G) TELEPHONE:  
(H) TELEFAX:

15 (ii) TITLE OF INVENTION: NOVEL VECTORS AND GENES EXHIBITING  
INCREASED EXPRESSION

(iii) NUMBER OF SEQUENCES: 11

20 (iv) CORRESPONDENCE ADDRESS:  
(A) ADDRESSEE: LAHIVE & COCKFIELD, LLP  
(B) STREET: 28 STATE STREET  
(C) CITY: BOSTON  
25 (D) STATE: MASSACHUSETTS  
(E) COUNTRY: US  
(F) ZIP: 02109

(v) COMPUTER READABLE FORM:  
30 (A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

35 (vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER: PCT/US98/  
(B) FILING DATE: 25 NOVEMBER 1998  
(C) CLASSIFICATION:

40 (vii) PRIOR APPLICATION DATA:  
(A) APPLICATION NUMBER: US 60/067,614  
(B) FILING DATE: 05 DECEMBER 1997  
(C) APPLICATION NUMBER: US 60/071,596  
(D) FILING DATE: 16 JANUARY 1998

45 (viii) ATTORNEY/AGENT INFORMATION:  
(A) NAME: REMILLARD, JANE E.  
(B) REGISTRATION NUMBER: 38,872  
(C) REFERENCE/DOCKET NUMBER: TTI-180PC

50 (ix) TELECOMMUNICATION INFORMATION:  
(A) TELEPHONE: (617)227-7400  
(B) TELEFAX: (617)742-4214

- 2 -

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 4374 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

10

## (ix) FEATURE:

- (A) NAME/KEY: CDS  
 (B) LOCATION: 1..4374

## 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATG GAA ATA GAG CTC TCC ACC TGC TTC TTT CTG TGC CTT TTG CGA TTC	48
Met Glu Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe	
1 5 10 15	
20 TGC TTT AGT GCC ACC AGA AGA TAC TAC CTG GGT GCA GTG GAA CTG TCA	96
Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser	
20 25 30	
25 TGG GAC TAT ATG CAA AGT GAT CTC GGA GAG CTG CCT GTG GAC GCA AGA	144
Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg	
35 40 45	
30 TTT CCT CCT CGC GTG CCA AAA TCT TTT CCA TTC AAC ACC TCA GTC GTG	192
Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val	
50 55 60	
TAC AAA AAG ACT CTG TTT GTA GAA TTC ACG GTT CAC CTT TTC AAC ATC	240
Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Val His Leu Phe Asn Ile	
35 65 70 75 80	
GCT AAG CCA AGG CCA CCC TGG ATG GGT CTG CTA GGT CCT ACC ATC CAA	288
Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln	
85 90 95	
40 GCT GAG GTT TAT GAT ACA GTG GTC ATT ACA CTT AAG AAC ATG GCT TCC	336
Ala Glu Val Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser	
100 105 110	
45 CAT CCT GTC TCC CTT CAT GCT GTT GGT GTA TCC TAC TGG AAA GCT TCT	384
His Pro Val Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser	
115 120 125	
GAG GGA GCT GAA TAT GAT GAT CAG ACC AGT CAA AGG GAG AAA GAA GAT	432
Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp	
130 135 140	
GAT AAA GTC TTC CCT GGT GGA AGC CAT ACA TAT GTC TGG CAA GTC CTG	480
Asp Lys Val Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu	
55 145 150 155 160	



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	AAA GAG AAT GGT CCA ATG GCC TCC GAC CCA CTG TGC CTT ACC TAC TCA	528
	Lys Glu Asn Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser	
	165 170 175	
5	TAT CTT TCT CAT GTG GAC CTG GTT AAA GAC TTG AAT TCA GGC CTC ATT	576
	Tyr Leu Ser His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile	
	180 185 190	
10	GGA GCC CTA CTA GTA TGT AGA GAA GGG AGT CTG GCC AAG GAA AAG ACA	624
	Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr	
	195 200 205	
15	CAG ACC TTG CAC AAA TTT ATA CTA CTT TTT GCT GTA TTT GAT GAA GGG	672
	Gln Thr Leu His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly	
	210 215 220	
20	AAA AGT TGG CAC TCA GAA ACA AAG AAC TCC CTC ATG CAA GAT AGG GAT	720
	Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp	
	225 230 235 240	
25	GCT GCA TCT GCT CGG GCC TGG CCT AAA ATG CAC ACA GTC AAT GGT TAT	768
	Ala Ala Ser Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr	
	245 250 255	
30	GTA AAC AGG AGC CTG CCA GGA CTG ATT GGA TGC CAC AGG AAA TCA GTC	816
	Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val	
	260 265 270	
35	TAT TGG CAT GTT ATA GGA ATG GGC ACC ACT CCT GAA GTG CAC TCA ATA	864
	Tyr Trp His Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile	
	275 280 285	
40	TTC CTC GAA GGA CAC ACA TTT CTT GTT AGA AAC CAT CGC CAG GCG TCC	912
	Phe Leu Glu Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser	
	290 295 300	
45	TTG GAA ATC TCG CCA ATA ACT TTC CTT ACT GCT CAA ACA CTC CTC ATG	960
	Leu Glu Ile Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met	
	305 310 315 320	
50	GAC CTT GGA CAG TTT CTA CTG TTT TGT CAT ATC TCT TCC CAC CAA CAT	1008
	Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His	
	325 330 335	
55	GAT GGC ATG GAA GCT TAT GTC AAA GTA GAC AGC TGT CCA GAG GAA CCC	1056
	Asp Gly Met Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro	
	340 345 350	
60	CAA CTA CGA ATG AAA AAT AAT GAA GAA GCG GAA GAC TAT GAT GAT GAT	1104
	Gln Leu Arg Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp	
	355 360 365	

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	CTT ACC GAT TCT GAA ATG GAT GTG GTC AGA TTT GAT GAT GAC AAC TCT	1152
	Leu Thr Asp Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser	
	370 375 380	
5	CCT TCC TTT ATC CAA ATT CGC TCA GTT GCC AAG AAG CAT CCT AAA ACT	1200
	Pro Ser Phe Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr	
	385 390 395 400	
10	TGG GTA CAT TAC ATT GCT GCT GAA GAG GAG GAC TGG GAC TAT GCT CCC	1248
	Trp Val His Tyr Ile Ala Ala Glu Glu Asp Trp Asp Tyr Ala Pro	
	405 410 415	
15	TTA GTC CTC GCC CCC GAT GAC AGA AGT TAT AAA AGT CAA TAT TTG AAC	1296
	Leu Val Leu Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn	
	420 425 430	
20	AAT GGC CCT CAG CGG ATT GGA AGG AAG TAC AAA AAA GTC CGA TTT ATG	1344
	Asn Gly Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met	
	435 440 445	
	GCA TAC ACA GAT GAA ACC TTT AAG ACT CGT GAA GCT ATT CAG CAT GAA	1392
	Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu	
	450 455 460	
25	TCA GGA ATC TTG GGA CCT TTA CTT TAT GGG GAA GTT GGA GAC ACA CTG	1440
	Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu	
	465 470 475 480	
30	CTC ATT ATA TTT AAG AAT CAA GCA AGC AGA CCA TAT AAC ATC TAC CCT	1488
	Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro	
	485 490 495	
35	CAC GGA ATC ACC GAT GTC CGT CCT TTG TAT TCA CGC AGA TTA CCA AAA	1536
	His Gly Ile Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys	
	500 505 510	
40	GGA GTA AAA CAT TTG AAG GAT TTT CCA ATT CTG CCC GGA GAA ATA TTC	1584
	Gly Val Lys His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe	
	515 520 525	
	AAA TAT AAA TGG ACA GTG ACT GTA GAA GAT GGG CCA ACT AAA TCA GAT	1632
	Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp	
	530 535 540	
45	CCT CGG TGC CTG ACC CGC TAT TAC TCT AGT TTC GTC AAT ATG GAG AGA	1680
	Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg	
	545 550 555 560	
50	GAT CTA GCT TCA GGA CTC ATT GGC CCT CTC CTC ATC TGC TAC AAA GAA	1728
	Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu	
	565 570 575	
55	TCT GTA GAT CAA AGA GGA AAC CAG ATA ATG TCA GAC AAG AGG AAT GTC	1776
	Ser Val Asp Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val	
	580 585 590	

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	ATC CTG TTT TCT GTA TTT GAT GAG AAC CGA AGC TGG TAC CTC ACA GAG	1824
	Ile Leu Phe Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu	
	595 600 605	
5	AAT ATA CAA CGC TTT CTC CCC AAT CCC GCT GGA GTG CAG CTT GAG GAT	1872
	Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp	
	610 615 620	
10	CCA GAG TTC CAA GCC TCC AAC ATC ATG CAC AGC ATC AAT GGC TAT GTT	1920
	Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val	
	625 630 635 640	
15	TTC GAT AGT TTG CAG TTG TCA GTT TGT TTG CAT GAA GTA GCA TAC TGG	1968
	Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp	
	645 650 655	
20	TAC ATT CTA AGC ATT GGA GCA CAG ACT GAC TTC CTT TCT GTC TTC TTC	2016
	Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe	
	660 665 670	
25	TCT GGA TAT ACC TTC AAA CAC AAA ATG GTC TAT GAA GAC ACA CTC ACC	2064
	Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr	
	675 680 685	
30	CTA TTC CCA TTC TCC GGA GAA ACT GTC TTC ATG TCG ATG GAA AAC CCA	2112
	Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro	
	690 695 700	
35	GGA CTA TGG ATT CTG GGG TGC CAC AAC TCA GAC TTT CGG AAC AGA GGC	2160
	Gly Leu Trp Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly	
	705 710 715 720	
40	ATG ACC GCC TTA CTG AAA GTT TCC AGT TGT GAC AAG AAC ACT GGA GAT	2208
	Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp	
	725 730 735	
45	TAT TAC GAG GAC AGT TAT GAA GAT ATT TCA GCA TAC TTG CTG AGT AAA	2256
	Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys	
	740 745 750	
50	AAC AAT GCC ATT GAA CCA AGA AGC TTC TCC CAG AAC CCA CCA GTC TTG	2304
	Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser Gln Asn Pro Pro Val Leu	
	755 760 765	
55	AAA CGC CAT CAA CGG GAA ATA ACT CGT ACT ACT CTT CAA TCA GAT CAA	2352
	Lys Arg His Gln Arg Glu Ile Thr Arg Thr Thr Leu Gln Ser Asp Gln	
	770 775 780	
60	GAG GAA ATT GAC TAT GAT GAT ACC ATA TCA GTT GAA ATG AAG AAG GAA	2400
	Glu Glu Ile Asp Tyr Asp Asp Thr Ile Ser Val Glu Met Lys Lys Glu	
	785 790 795 800	

- 6 -

	GAT TTC GAC ATT TAT GAT GAG GAT GAA AAT CAG AGC CCC CGC AGC TTT	2448
	Asp Phe Asp Ile Tyr Asp Glu Asp Glu Asn Gln Ser Pro Arg Ser Phe	
	805 810 815	
5	CAA AAG AAA ACA CGA CAC TAT TTT ATT GCT GCA GTG GAG AGG CTC TGG	2496
	Gln Lys Lys Thr Arg His Tyr Phe Ile Ala Ala Val Glu Arg Leu Trp	
	820 825 830	
10	GAT TAT GGG ATG AGT AGC TCC CCA CAT GTT CTA AGA AAC AGG GCT CAG	2544
	Asp Tyr Gly Met Ser Ser Ser Pro His Val Leu Arg Asn Arg Ala Gln	
	835 840 845	
15	AGT GGC AGT GTC CCT CAG TTC AAG AAA GTA GTA TTC CAG GAA TTT ACC	2592
	Ser Gly Ser Val Pro Gln Phe Lys Lys Val Val Phe Gln Glu Phe Thr	
	850 855 860	
20	GAT GGC TCC TTT ACT CAA CCC TTA TAC CGT GGA GAA CTA AAT GAA CAT	2640
	Asp Gly Ser Phe Thr Gln Pro Leu Tyr Arg Gly Glu Leu Asn Glu His	
	865 870 875 880	
	TTG GGA CTC CTG GGG CCA TAT ATA AGA GCA GAA GTT GAA GAT AAT ATC	2688
	Leu Gly Leu Leu Gly Pro Tyr Ile Arg Ala Glu Val Glu Asp Asn Ile	
	885 890 895	
25	ATG GTT ACC TTC AGA AAT CAG GCC TCT CGT CCC TAT TCC TTC TAT TCT	2736
	Met Val Thr Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser	
	900 905 910	
30	TCC CTC ATA TCA TAT GAG GAA GAT CAG AGG CAA GGA GCA GAA CCT AGA	2784
	Ser Leu Ile Ser Tyr Glu Glu Asp Gln Arg Gln Gly Ala Glu Pro Arg	
	915 920 925	
35	AAA AAC TTT GTC AAG CCT AAT GAA ACC AAA ACT TAC TTT TGG AAA GTG	2832
	Lys Asn Phe Val Lys Pro Asn Glu Thr Lys Thr Tyr Phe Trp Lys Val	
	930 935 940	
40	CAA CAT CAT ATG GCA CCC ACT AAA GAT GAG TTT GAC TGC AAA GCC TGG	2880
	Gln His His Met Ala Pro Thr Lys Asp Glu Phe Asp Cys Lys Ala Trp	
	945 950 955 960	
	GCT TAT TTC TCC GAT GTC GAC CTG GAA AAA GAT GTG CAC TCA GGC CTG	2928
	Ala Tyr Phe Ser Asp Val Asp Leu Glu Lys Asp Val His Ser Gly Leu	
	965 970 975	
45	ATT GGA CCC CTT CTG GTC TGC CAC ACC AAC ACA CTG AAC CCT GCT CAT	2976
	Ile Gly Pro Leu Leu Val Cys His Thr Asn Thr Leu Asn Pro Ala His	
	980 985 990	
50	GGG AGA CAA GTG ACA GTA CAG GAA TTT GCT CTG TTT TTC ACC ATC TTC	3024
	Gly Arg Gln Val Thr Val Gln Glu Phe Ala Leu Phe Thr Ile Phe	
	995 1000 1005	
55	GAT GAG ACC AAA AGC TGG TAC TTC ACT GAA AAT ATG GAA AGA AAC TGC	3072
	Asp Glu Thr Lys Ser Trp Tyr Phe Thr Glu Asn Met Glu Arg Asn Cys	
	1010 1015 1020	

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5	AGG GCT CCC TGC AAT ATC CAG ATG GAA GAT CCC ACT TTT AAA GAG AAT	3120
	Arg Ala Pro Cys Asn Ile Gln Met Glu Asp Pro Thr Phe Lys Glu Asn	
	1025 1030 1035 1040	
	TAT CGC TTC CAT GCA ATC AAT GGC TAC ATA ATG GAT ACA CTA CCT GGC	3168
	Tyr Arg Phe His Ala Ile Asn Gly Tyr Ile Met Asp Thr Leu Pro Gly	
10	1045 1050 1055	
	TTA GTA ATG GCT CAG GAT CAA AGG ATT CGA TGG TAT CTG CTC AGC ATG	3216
	Leu Val Met Ala Gln Asp Gln Arg Ile Arg Trp Tyr Leu Leu Ser Met	
	1060 1065 1070	
	GGC AGC AAT GAA AAC ATC CAT TCT ATT CAT TTC TCC GGA CAT GTG TTC	3264
15	Gly Ser Asn Glu Asn Ile His Ser Ile His Phe Ser Gly His Val Phe	
	1075 1080 1085	
	ACT GTA CGA AAA AAA GAG GAG TAT AAA ATG GCA CTG TAC AAT CTC TAT	3312
	Thr Val Arg Lys Lys Glu Glu Tyr Lys Met Ala Leu Tyr Asn Leu Tyr	
	1090 1095 1100	
20	CCC GGA GTT TTC GAG ACA GTG GAA ATG TTA CCA TCC AAA GCT GGA ATT	3360
	Pro Gly Val Phe Glu Thr Val Glu Met Leu Pro Ser Lys Ala Gly Ile	
	1105 1110 1115 1120	
	TGG CGG GTG GAA TGC CTT ATT GGC GAG CAT CTA CAT GCT GGG ATG AGC	3408
	Trp Arg Val Glu Cys Leu Ile Gly Glu His Leu His Ala Gly Met Ser	
25	1125 1130 1135	
	ACA CTT TTT CTG GTG TAC TCC AAT AAG TGT CAG ACT CCC CTG GGA ATG	3456
	Thr Leu Phe Leu Val Tyr Ser Asn Lys Cys Gln Thr Pro Leu Gly Met	
	1140 1145 1150	
	GCT TCT GGA CAC ATT AGA GAT TTT CAG ATT ACA GCT TCA GGA CAA TAT	3504
30	Ala Ser Gly His Ile Arg Asp Phe Gln Ile Thr Ala Ser Gly Gln Tyr	
	1155 1160 1165	
	GGA CAG TGG GCC CCA AAG CTG GCC AGA CTT CAT TAT TCC GGA TCA ATC	3552
	Gly Gln Trp Ala Pro Lys Leu Ala Arg Leu His Tyr Ser Gly Ser Ile	
	1170 1175 1180	
35	AAT GCC TGG AGC ACC AAG GAG CCC TTT TCT TGG ATC AAA GTT GAC CTG	3600
	Asn Ala Trp Ser Thr Lys Glu Pro Phe Ser Trp Ile Lys Val Asp Leu	
	1185 1190 1195 1200	
	TTG GCA CCA ATG ATT ATT CAC GGC ATC AAG ACC CAG GGT GCC CGT CAG	3648
	Leu Ala Pro Met Ile Ile His Gly Ile Lys Thr Gln Gly Ala Arg Gln	
40	1205 1210 1215	
	AAG TTC TCC AGC CTC TAC ATC TCT CAA TTT ATC ATC ATG TAT AGT CTC	3696
	Lys Phe Ser Ser Leu Tyr Ile Ser Gln Phe Ile Ile Met Tyr Ser Leu	
	1220 1225 1230	

- 8 -

	GAT GGG AAG AAG TGG CAG ACT TAT CGA GGA AAT TCC ACT GGA ACC CTC	3744
	Asp Gly Lys Lys Trp Gln Thr Tyr Arg Gly Asn Ser Thr Gly Thr Leu	
	1235 1240 1245	
5	ATG GTC TTC TTT GGC AAT GTG GAT TCA TCT GGG ATA AAA CAC AAT ATT	3792
	Met Val Phe Phe Gly Asn Val Asp Ser Ser Gly Ile Lys His Asn Ile	
	1250 1255 1260	
10	TTC AAC CCT CCA ATT ATT GCT CGA TAC ATC CGT TTG CAC CCA ACT CAT	3840
	Phe Asn Pro Pro Ile Ile Ala Arg Tyr Ile Arg Leu His Pro Thr His	
	1265 1270 1275 1280	
15	TAT AGC ATT CGC AGC ACT CTT CGC ATG GAG TTG ATG GGC TGT GAT TTA	3888
	Tyr Ser Ile Arg Ser Thr Leu Arg Met Glu Leu Met Gly Cys Asp Leu	
	1285 1290 1295	
20	AAT AGT TGC AGC ATG CCA TTG GGA ATG GAG AGT AAA GCA ATA TCA GAT	3936
	Asn Ser Cys Ser Met Pro Leu Gly Met Glu Ser Lys Ala Ile Ser Asp	
	1300 1305 1310	
25	GCA CAG ATT ACT GCT TCA TCC TAC TTT ACC AAT ATG TTT GCC ACC TGG	3984
	Ala Gln Ile Thr Ala Ser Ser Tyr Phe Thr Asn Met Phe Ala Thr Trp	
	1315 1320 1325	
30	TCT CCT TCA AAA GCT CGA CTA CAC CTA CAA GGG AGG AGT AAT GCC TGG	4032
	Ser Pro Ser Lys Ala Arg Leu His Leu Gln Gly Arg Ser Asn Ala Trp	
	1330 1335 1340	
35	AGA CCT CAA GTT AAC AAT CCA AAA GAG TGG CTG CAA GTG GAC TTC CAG	4080
	Arg Pro Gln Val Asn Asn Pro Lys Glu Trp Leu Gln Val Asp Phe Gln	
	1345 1350 1355 1360	
40	AAG ACA ATG AAA GTC ACA GGA GTA ACT ACT CAG GGA GTA AAA TCT CTG	4128
	Lys Thr Met Lys Val Thr Gly Val Thr Thr Gln Gly Val Lys Ser Leu	
	1365 1370 1375	
45	CTT ACC TCT ATG TAC GTG AAG GAG TTC CTC ATA TCG TCG TCG CAA GAT	4176
	Leu Thr Ser Met Tyr Val Lys Glu Phe Leu Ile Ser Ser Ser Gln Asp	
	1380 1385 1390	
50	GGC CAT CAG TGG ACT CTC TTT TTT CAA AAT GGC AAA GTA AAA GTT TTC	4224
	Gly His Gln Trp Thr Leu Phe Phe Gln Asn Gly Lys Val Lys Val Phe	
	1395 1400 1405	
55	CAG GGA AAT CAA GAC TCC TTC ACA CCT GTC GTG AAC TCT CTA GAC CCA	4272
	Gln Gly Asn Gln Asp Ser Phe Thr Pro Val Val Asn Ser Leu Asp Pro	
	1410 1415 1420	
55	CCG TTA CTC ACT CGC TAC CTT CGA ATT CAC CCC CAG AGT TGG GTG CAC	4320
	Pro Leu Leu Thr Arg Tyr Leu Arg Ile His Pro Gln Ser Trp Val His	
	1425 1430 1435 1440	
55	CAG ATT GCC CTG AGG ATG GAG GTT CTG GGC TGC GAG GCA CAG GAC CTC	4368
	Gln Ile Ala Leu Arg Met Glu Val Leu Gly Cys Glu Ala Gln Asp Leu	
	1445 1450 1455	

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TAC TG  
Tyr

4374

## 5 (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 9164 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## 15 (ix) FEATURE:

- (A) NAME/KEY: CDS  
(B) LOCATION: 1006..5376

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

20 GTCGACGGTA TCGATAAGCT TGATATCGAA TTCCTGCAGC CCGGGGGATC CACTAGTACT 60  
CGAGACCTAG GAGTTAATTT TTAAAAAGCA GTCAAAAGTC CAAGTGGCCC TTGCGAGCAT 120  
25 TTACTCTCTC TGTTCGCTCT GGTAAATAAT CTCAGGAGCA CAAACATTCC TTACTAGTCC 180  
TAGAAGTTAA TTTTAAAAA GCAGTCAAAA GTCCAAGTGG CCCTTGCGAG CATTTACTCT 240  
CTCTGTTTGC TCTGGTTAAT AATCTCAGGA GCACAAACAT TCCTTACTAG TTCTAGAGCG 300  
30 GCCGCCAGTG TGCTGGAATT CGGCTTTTTT AGGGCTGGAA GCTACCTTTG ACATCATTTT 360  
CTCTGCGAAT GCATGTATAA TTTCTACAGA ACCTATTAGA AAGGATCACC CAGCCTCTGC 420  
35 TTTTGTACAA CTTTCCCTTA AAAAAGTACC AATCCACTG CTGTTTGGCC CAATAGTGAG 480  
AACTTTTTCC TGCTGCCTCT TGGTGCTTTT GCCTATGGCC CCTATTCTGC CTGCTGAAGA 540  
CACTCTTGCC AGCATGGACT TAAACCCCTC CAGCTCTGAC AATCCTCTTT CTCTTTTGGT 600  
40 TTACATGAAG GGTCTGGCAG CCAAAGCAAT CACTCAAAGT TCAAACCTTA TCATTTTTTG 660  
CTTGTTTCCT CTGGCCTTG GTTTTGTACA TCAGCTTTGA AAATACCATC CCAGGGTTAA 720  
45 TGCTGGGGTT AATTTATAAC TAAGAGTGCT CTAGTTTTCG AATACAGGAC ATGCTATAAA 780  
AATGGAAAGA TGTTCCTTTC TGAGAGATCT CGAGGAAGCT AACACAAAG AACACAAAC 840  
AACAAATCAGG TAAGTATCCT TTTTACAGCA CAACTTAATG AGACAGATAG AAAGTGGTCT 900  
50 TGTAGAAACA GAGTAGTCGC CTGCTTTTCT GCCAGGTGCT GACTTCTCTC CCCTTCTCTT 960  
TTTTCCTTTT CTCAGGATAA CAAGAACGAA ACAATAACAG CCACC ATG GAA ATA 1014  
Met Glu Ile

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	GAG CTC TCC ACC TGC TTC TTT CTG TGC CTT TTG CGA TTC TGC TTT AGT	1062
	Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe Cys Phe Ser	
	5 10 15	
5	GCC ACC AGA AGA TAC TAC CTG GGT GCA GTG GAA CTG TCA TGG GAC TAT	1110
	Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser Trp Asp Tyr	
	20 25 30 35	
10	ATG CAA AGT GAT CTC GGT GAG CTG CCT GTG GAC GCA AGA TTT CCT CCT	1158
	Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg Phe Pro Pro	
	40 45 50	
	AGA GTG CCA AAA TCT TTT CCA TTC AAC ACC TCA GTC GTG TAC AAA AAG	1206
15	Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val Tyr Lys Lys	
	55 60 65	
	ACT CTG TTT GTA GAA TTC ACG GTT CAC CTT TTC AAC ATC GCT AAG CCA	1254
20	Thr Leu Phe Val Glu Phe Thr Val His Leu Phe Asn Ile Ala Lys Pro	
	70 75 80	
	AGG CCA CCC TGG ATG GGT CTG CTA GGT CCT ACC ATC CAG GCT GAG GTT	1302
	Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln Ala Glu Val	
	85 90 95	
25	TAT GAT ACA GTG GTC ATT ACA CTT AAG AAC ATG GCT TCC CAT CCT GTC	1350
	Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser His Pro Val	
	100 105 110 115	
30	AGT CTT CAT GCT GTT GGT GTA TCC TAC TGG AAA GCT TCT GAG GGA GCT	1398
	Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser Glu Gly Ala	
	120 125 130	
	GAA TAT GAT GAT CAG ACC AGT CAA AGG GAG AAA GAA GAT GAT AAA GTC	1446
35	Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp Asp Lys Val	
	135 140 145	
	TTC CCT GGT GGA AGC CAT ACA TAT GTC TGG CAG GTC CTG AAA GAG AAT	1494
40	Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu Lys Glu Asn	
	150 155 160	
	GGT CCA ATG GCC TCT GAC CCA CTG TGC CTT ACC TAC TCA TAT CTT TCT	1542
	Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser Tyr Leu Ser	
	165 170 175	
45	CAT GTG GAC CTG GTA AAA GAC TTG AAT TCA GGC CTC ATT GGA GCC CTA	1590
	His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile Gly Ala Leu	
	180 185 190 195	
50	CTA GTA TGT AGA GAA GGG AGT CTG GCC AAG GAA AAG ACA CAG ACC TTG	1638
	Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr Gln Thr Leu	
	200 205 210	



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	CAC AAA TTT ATA CTA CTT TTT GCT GTA TTT GAT GAA GGG AAA AGT TGG	1686
	His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly Lys Ser Trp	
	215 220 225	
5	CAC TCA GAA ACA AAG AAC TCC TTG ATG CAG GAT AGG GAT GCT GCA TCT	1734
	His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp Ala Ala Ser	
	230 235 240	
10	GCT CGG GCC TGG CCT AAA ATG CAC ACA GTC AAT GGT TAT GTA AAC AGG	1782
	Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr Val Asn Arg	
	245 250 255	
15	TCT CTG CCA GGT CTG ATT GGA TGC CAC AGG AAA TCA GTC TAT TGG CAT	1830
	Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val Tyr Trp His	
	260 265 270 275	
20	GTG ATT GGA ATG GGC ACC ACT CCT GAA GTG CAC TCA ATA TTC CTC GAA	1878
	Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile Phe Leu Glu	
	280 285 290	
	GGT CAC ACA TTT CTT GTG AGG AAC CAT CGC CAG GCG TCC TTG GAA ATC	1926
	Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser Leu Glu Ile	
	295 300 305	
25	TCG CCA ATA ACT TTC CTT ACT GCT CAA ACA CTC TTG ATG GAC CTT GGA	1974
	Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met Asp Leu Gly	
	310 315 320	
30	CAG TTT CTA CTG TTT TGT CAT ATC TCT TCC CAC CAA CAT GAT GGC ATG	2022
	Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His Asp Gly Met	
	325 330 335	
35	GAA GCT TAT GTC AAA GTA GAC AGC TGT CCA GAG GAA CCC CAA CTA CGA	2070
	Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro Gln Leu Arg	
	340 345 350 355	
40	ATG AAA AAT AAT GAA GAA GCG GAA GAC TAT GAT GAT GAT CTT ACT GAT	2118
	Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp Leu Thr Asp	
	360 365 370	
	TCT GAA ATG GAT GTG GTC AGG TTT GAT GAT GAC AAC TCT CCT TCC TTT	2166
	Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser Pro Ser Phe	
	375 380 385	
45	ATC CAA ATT CGC TCA GTT GCC AAG AAG CAT CCT AAA ACT TGG GTA CAT	2214
	Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr Trp Val His	
	390 395 400	
50	TAC ATT GCT GCT GAA GAG GAG GAC TGG GAC TAT GCT CCC TTA GTC CTC	2262
	Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro Leu Val Leu	
	405 410 415	
55	GCC CCC GAT GAC AGA AGT TAT AAA AGT CAA TAT TTG AAC AAT GGC CCT	2310
	Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn Asn Gly Pro	
	420 425 430 435	

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	CAG CGG ATT GGT AGG AAG TAC AAA AAA GTC CGA TTT ATG GCA TAC ACA	2358
	Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met Ala Tyr Thr	
	440 445 450	
5	GAT GAA ACC TTT AAG ACT CGT GAA GCT ATT CAG CAT GAA TCA GGA ATC	2406
	Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu Ser Gly Ile	
	455 460 465	
10	TTG GGA CCT TTA CTT TAT GGG GAA GTT GGA GAC ACA CTG TTG ATT ATA	2454
	Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu Leu Ile Ile	
	470 475 480	
15	TTT AAG AAT CAA GCA AGC AGA CCA TAT AAC ATC TAC CCT CAC GGA ATC	2502
	Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro His Gly Ile	
	485 490 495	
20	ACT GAT GTC CGT CCT TTG TAT TCA AGG AGA TTA CCA AAA GGT GTA AAA	2550
	Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys Gly Val Lys	
	500 505 510 515	
	CAT TTG AAG GAT TTT CCA ATT CTG CCA GGA GAA ATA TTC AAA TAT AAA	2598
	His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe Lys Tyr Lys	
	520 525 530	
25	TGG ACA GTG ACT GTA GAA GAT GGG CCA ACT AAA TCA GAT CCT CGG TGC	2646
	Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp Pro Arg Cys	
	535 540 545	
30	CTG ACC CGC TAT TAC TCT AGT TTC GTT AAT ATG GAG AGA GAT CTA GCT	2694
	Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg Asp Leu Ala	
	550 555 560	
35	TCA GGA CTC ATT GGC CCT CTC CTC ATC TGC TAC AAA GAA TCT GTA GAT	2742
	Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu Ser Val Asp	
	565 570 575	
40	CAA AGA GGA AAC CAG ATA ATG TCA GAC AAG AGG AAT GTC ATC CTG TTT	2790
	Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val Ile Leu Phe	
	580 585 590 595	
	TCT GTA TTT GAT GAG AAC CGA AGC TGG TAC CTC ACA GAG AAT ATA CAA	2838
	Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu Asn Ile Gln	
	600 605 610	
45	CGC TTT CTC CCC AAT CCA GCT GGA GTG CAG CTT GAG GAT CCA GAG TTC	2886
	Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp Pro Glu Phe	
	615 620 625	
50	CAA GCC TCC AAC ATC ATG CAC AGC ATC AAT GGC TAT GTT TTT GAT AGT	2934
	Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val Phe Asp Ser	
	630 635 640	

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	TTG CAG TTG TCA GTT TGT TTG CAT GAG GTG GCA TAC TGG TAC ATT CTA	2982
	Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp Tyr Ile Leu	
	645 650 655	
5	AGC ATT GGA GCA CAG ACT GAC TTC CTT TCT GTC TTC TTC TCT GGA TAT	3030
	Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe Ser Gly Tyr	
	660 665 670 675	
10	ACC TTC AAA CAC AAA ATG GTC TAT GAA GAC ACA CTC ACC CTA TTC CCA	3078
	Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr Leu Phe Pro	
	680 685 690	
15	TTC TCA GGA GAA ACT GTC TTC ATG TCG ATG GAA AAC CCA GGT CTA TGG	3126
	Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro Gly Leu Trp	
	695 700 705	
20	ATT CTG GGG TGC CAC AAC TCA GAC TTT CGG AAC AGA GGC ATG ACC GCC	3174
	Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly Met Thr Ala	
	710 715 720	
25	TTA CTG AAG GTT TCT AGT TGT GAC AAG AAC ACT GGT GAT TAT TAC GAG	3222
	Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp Tyr Tyr Glu	
	725 730 735	
30	GAC AGT TAT GAA GAT ATT TCA GCA TAC TTG CTG AGT AAA AAC AAT GCC	3270
	Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys Asn Asn Ala	
	740 745 750 755	
35	ATT GAA CCA AGA AGC TTC TCC CAG AAC CCA CCA GTC TTG AAA CGC CAT	3318
	Ile Glu Pro Arg Ser Phe Ser Gln Asn Pro Pro Val Leu Lys Arg His	
	760 765 770	
40	CAA CGG GAA ATA ACT CGT ACT ACT CTT CAG TCA GAT CAA GAG GAA ATT	3366
	Gln Arg Glu Ile Thr Arg Thr Thr Leu Gln Ser Asp Gln Glu Glu Ile	
	775 780 785	
45	GAC TAT GAT GAT ACC ATA TCA GTT GAA ATG AAG AAG GAA GAT TTT GAC	3414
	Asp Tyr Asp Asp Thr Ile Ser Val Glu Met Lys Lys Glu Asp Phe Asp	
	790 795 800	
50	ATT TAT GAT GAG GAT GAA AAT CAG AGC CCC CGC AGC TTT CAA AAG AAA	3462
	Ile Tyr Asp Glu Asp Glu Asn Gln Ser Pro Arg Ser Phe Gln Lys Lys	
	805 810 815	
55	ACA CGA CAC TAT TTT ATT GCT GCA GTG GAG AGG CTC TGG GAT TAT GGG	3510
	Thr Arg His Tyr Phe Ile Ala Ala Val Glu Arg Leu Trp Asp Tyr Gly	
	820 825 830 835	
60	ATG AGT AGC TCC CCA CAT GTT CTA AGA AAC AGG GCT CAG AGT GGC AGT	3558
	Met Ser Ser Ser Pro His Val Leu Arg Asn Arg Ala Gln Ser Gly Ser	
	840 845 850	
65	GTC CCT CAG TTC AAG AAA GTT GTT TTC CAG GAA TTT ACT GAT GGC TCC	3606
	Val Pro Gln Phe Lys Lys Val Val Phe Gln Glu Phe Thr Asp Gly Ser	
	855 860 865	

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	TTT ACT CAG CCC TTA TAC CGT GGA GAA CTA AAT GAA CAT TTG GGA CTC	3654
	Phe Thr Gln Pro Leu Tyr Arg Gly Glu Leu Asn Glu His Leu Gly Leu	
	870 875 880	
5	CTG GGG CCA TAT ATA AGA GCA GAA GTT GAA GAT AAT ATC ATG GTA ACT	3702
	Leu Gly Pro Tyr Ile Arg Ala Glu Val Glu Asp Asn Ile Met Val Thr	
	885 890 895	
10	TTC AGA AAT CAG GCC TCT CGT CCC TAT TCC TTC TAT TCT AGC CTT ATT	3750
	Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser Ser Leu Ile	
	900 905 910 915	
15	TCT TAT GAG GAA GAT CAG AGG CAA GGA GCA GAA CCT AGA AAA AAC TTT	3798
	Ser Tyr Glu Glu Asp Gln Arg Gln Gly Ala Glu Pro Arg Lys Asn Phe	
	920 925 930	
20	GTC AAG CCT AAT GAA ACC AAA ACT TAC TTT TGG AAA GTG CAA CAT CAT	3846
	Val Lys Pro Asn Glu Thr Lys Thr Tyr Phe Trp Lys Val Gln His His	
	935 940 945	
	ATG GCA CCC ACT AAA GAT GAG TTT GAC TGC AAA GCC TGG GCT TAT TTC	3894
	Met Ala Pro Thr Lys Asp Glu Phe Asp Cys Lys Ala Trp Ala Tyr Phe	
	950 955 960	
25	TCT GAT GTT GAC CTG GAA AAA GAT GTG CAC TCA GGC CTG ATT GGA CCC	3942
	Ser Asp Val Asp Leu Glu Lys Asp Val His Ser Gly Leu Ile Gly Pro	
	965 970 975	
30	CTT CTG GTC TGC CAC ACT AAC ACA CTG AAC CCT GCT CAT GGG AGA CAA	3990
	Leu Leu Val Cys His Thr Asn Thr Leu Asn Pro Ala His Gly Arg Gln	
	980 985 990 995	
35	GTG ACA GTA CAG GAA TTT GCT CTG TTT TTC ACC ATC TTT GAT GAG ACC	4038
	Val Thr Val Gln Glu Phe Ala Leu Phe Phe Thr Ile Phe Asp Glu Thr	
	1000 1005 1010	
40	AAA AGC TGG TAC TTC ACT GAA AAT ATG GAA AGA AAC TGC AGG GCT CCC	4086
	Lys Ser Trp Tyr Phe Thr Glu Asn Met Glu Arg Asn Cys Arg Ala Pro	
	1015 1020 1025	
	TGC AAT ATC CAG ATG GAA GAT CCC ACT TTT AAA GAG AAT TAT CGC TTC	4134
	Cys Asn Ile Gln Met Glu Asp Pro Thr Phe Lys Glu Asn Tyr Arg Phe	
	1030 1035 1040	
45	CAT GCA ATC AAT GGC TAC ATA ATG GAT ACA CTA CCT GGC TTA GTA ATG	4182
	His Ala Ile Asn Gly Tyr Ile Met Asp Thr Leu Pro Gly Leu Val Met	
	1045 1050 1055	
50	GCT CAG GAT CAA AGG ATT CGA TGG TAT CTG CTC AGC ATG GGC AGC AAT	4230
	Ala Gln Asp Gln Arg Ile Arg Trp Tyr Leu Leu Ser Met Gly Ser Asn	
	1060 1065 1070 1075	

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	GAA AAC ATC CAT TCT ATT CAT TTC AGT GGA CAT GTG TTC ACT GTA CGA	4278
	Glu Asn Ile His Ser Ile His Phe Ser Gly His Val Phe Thr Val Arg	
	1080 1085 1090	
5	AAA AAA GAG GAG TAT AAA ATG GCA CTG TAC AAT CTC TAT CCA GGT GTT	4326
	Lys Lys Glu Glu Tyr Lys Met Ala Leu Tyr Asn Leu Tyr Pro Gly Val	
	1095 1100 1105	
10	TTT GAG ACA GTG GAA ATG TTA CCA TCC AAA GCT GGA ATT TGG CGG GTG	4374
	Phe Glu Thr Val Glu Met Leu Pro Ser Lys Ala Gly Ile Trp Arg Val	
	1110 1115 1120	
15	GAA TGC CTT ATT GGC GAG CAT CTA CAT GCT GGG ATG AGC ACA CTT TTT	4422
	Glu Cys Leu Ile Gly Glu His Leu His Ala Gly Met Ser Thr Leu Phe	
	1125 1130 1135	
20	CTG GTG TAC AGC AAT AAG TGT CAG ACT CCC CTG GGA ATG GCT TCT GGA	4470
	Leu Val Tyr Ser Asn Lys Cys Gln Thr Pro Leu Gly Met Ala Ser Gly	
	1140 1145 1150 1155	
	CAC ATT AGA GAT TTT CAG ATT ACA GCT TCA GGA CAA TAT GGA CAG TGG	4518
	His Ile Arg Asp Phe Gln Ile Thr Ala Ser Gly Gln Tyr Gly Gln Trp	
	1160 1165 1170	
25	GCC CCA AAG CTG GCC AGA CTT CAT TAT TCC GGA TCA ATC AAT GCC TGG	4566
	Ala Pro Lys Leu Ala Arg Leu His Tyr Ser Gly Ser Ile Asn Ala Trp	
	1175 1180 1185	
30	AGC ACC AAG GAG CCC TTT TCT TGG ATC AAG GTG GAT CTG TTG GCA CCA	4614
	Ser Thr Lys Glu Pro Phe Ser Trp Ile Lys Val Asp Leu Leu Ala Pro	
	1190 1195 1200	
35	ATG ATT ATT CAC GGC ATC AAG ACC CAG GGT GCC CGT CAG AAG TTC TCC	4662
	Met Ile Ile His Gly Ile Lys Thr Gln Gly Ala Arg Gln Lys Phe Ser	
	1205 1210 1215	
40	AGC CTC TAC ATC TCT CAG TTT ATC ATC ATG TAT AGT CTT GAT GGG AAG	4710
	Ser Leu Tyr Ile Ser Gln Phe Ile Ile Met Tyr Ser Leu Asp Gly Lys	
	1220 1225 1230 1235	
	AAG TGG CAG ACT TAT CGA GGA AAT TCC ACT GGA ACC TTA ATG GTC TTC	4758
	Lys Trp Gln Thr Tyr Arg Gly Asn Ser Thr Gly Thr Leu Met Val Phe	
	1240 1245 1250	
45	TTT GGC AAT GTG GAT TCA TCT GGG ATA AAA CAC AAT ATT TTT AAC CCT	4806
	Phe Gly Asn Val Asp Ser Ser Gly Ile Lys His Asn Ile Phe Asn Pro	
	1255 1260 1265	
50	CCA ATT ATT GCT CGA TAC ATC CGT TTG CAC CCA ACT CAT TAT AGC ATT	4854
	Pro Ile Ile Ala Arg Tyr Ile Arg Leu His Pro Thr His Tyr Ser Ile	
	1270 1275 1280	
55	CGC AGC ACT CTT CGC ATG GAG TTG ATG GGC TGT GAT TTA AAT AGT TGC	4902
	Arg Ser Thr Leu Arg Met Glu Leu Met Gly Cys Asp Leu Asn Ser Cys	
	1285 1290 1295	

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	AGC ATG CCA TTG GGA ATG GAG AGT AAA GCA ATA TCA GAT GCA CAG ATT	4950
	Ser Met Pro Leu Gly Met Glu Ser Lys Ala Ile Ser Asp Ala Gln Ile	
	1300 1305 1310 1315	
5	ACT GCT TCA TCC TAC TTT ACC AAT ATG TTT GCC ACC TGG TCT CCT TCA	4998
	Thr Ala Ser Ser Tyr Phe Thr Asn Met Phe Ala Thr Trp Ser Pro Ser	
	1320 1325 1330	
10	AAA GCT CGA CTT CAC CTC CAA GGG AGG AGT AAT GCC TGG AGA CCT CAG	5046
	Lys Ala Arg Leu His Leu Gln Gly Arg Ser Asn Ala Trp Arg Pro Gln	
	1335 1340 1345	
	GTG AAT AAT CCA AAA GAG TGG CTG CAA GTG GAC TTC CAG AAG ACA ATG	5094
15	Val Asn Asn Pro Lys Glu Trp Leu Gln Val Asp Phe Gln Lys Thr Met	
	1350 1355 1360	
	AAA GTC ACA GGA GTA ACT ACT CAG GGA GTA AAA TCT CTG CTT ACC AGC	5142
20	Lys Val Thr Gly Val Thr Thr Gln Gly Val Lys Ser Leu Leu Thr Ser	
	1365 1370 1375	
	ATG TAT GTG AAG GAG TTC CTC ATC TCC AGC AGT CAA GAT GGC CAT CAG	5190
	Met Tyr Val Lys Glu Phe Leu Ile Ser Ser Ser Gln Asp Gly His Gln	
	1380 1385 1390 1395	
25	TGG ACT CTC TTT TTT CAG AAT GGC AAA GTA AAG GTT TTT CAG GGA AAT	5238
	Trp Thr Leu Phe Phe Gln Asn Gly Lys Val Lys Val Phe Gln Gly Asn	
	1400 1405 1410	
30	CAA GAC TCC TTC ACA CCT GTG GTG AAC TCT CTA GAC CCA CCG TTA CTG	5286
	Gln Asp Ser Phe Thr Pro Val Val Asn Ser Leu Asp Pro Pro Leu Leu	
	1415 1420 1425	
	ACT CGC TAC CTT CGA ATT CAC CCC CAG AGT TGG GTG CAC CAG ATT GCC	5334
35	Thr Arg Tyr Leu Arg Ile His Pro Gln Ser Trp Val His Gln Ile Ala	
	1430 1435 1440	
	CTG AGG ATG GAG GTT CTG GGC TGC GAG GCA CAG GAC CTC TAC	5376
40	Leu Arg Met Glu Val Leu Gly Cys Glu Ala Gln Asp Leu Tyr	
	1445 1450 1455	
	TGAGGGTGGC CACTGCAGCA CCTGCCACTG CCGTCACCTC TCCCTCCTCA GCTCCAGGGC	5436
	AGTGTCCTCCTC CCTGGCTTGC CTTCTACCTT TGTGCTAAAT CCTAGCAGAC ACTGCCTTGA	5496
45	AGCCTCCTGA ATTAACATATC ATCAGTCCTG CATTTCTTTG GTGGGGGGCC AGGAGGGTGC	5556
	ATCCAATTTA ACTTAACCTCT TACCTATTTT CTGCAGCTGC TCCCAGATTA CTCCTTCCTT	5616
50	CCAATATAAC TAGGCAAAAA GAAGTGAGGA GAAACCTGCA TGAAAGCATT CTTCCTTGAA	5676
	AAGTTAGGCC TCTCAGAGTC ACCACTTCCT CTGTTGTAGA AAAACTATGT GATGAAACTT	5736
55	TGAAAAAGAT ATTTATGATG TTAACCTGTT TATTGCAGCT TATAATGGTT ACAAATAAAG	5796

	CAATAGCATC	ACAAATTTCA	CAAATAAAGC	ATTTTTTTCA	CTGCATTCTA	GTTGTGGTTT	5856
	GTCCAAACTC	ATCAATGTAT	CTTATCATGT	CTGGATCCCC	GGGTGGCATC	CCTGTGACCC	5916
5	CTCCCCAGTG	CCTCTCCTGG	CCCTGGAAGT	TGCCACTCCA	GTGCCCACCA	GCCTTGTCTT	5976
	AATAAAATTA	AGTTGCATCA	TTTTGTCTGA	CTAGGTGTCC	TTCTATAATA	TTATGGGGTG	6036
	GAGGGGGGTG	GTATGGAGCA	AGGGGCAAGT	TGGGAAGACA	ACCTGTAGGG	CCTGCGGGGT	6096
10	CTATTGCGGA	ACCAAGCTGG	AGTGCAGTGG	CACAATCTTG	GCTCACTGCA	ATCTCCGCCT	6156
	CCTGGGTTCA	AGCGATTCTC	CTGCCTCAGC	CTCCCGAGTT	GTTGGGATTC	CAGGCATGCA	6216
15	TGACCAGGCT	CAGCTAATTT	TTGTTTTTTT	GGTAGAGACG	GGGTTTCACC	ATATTGGCCA	6276
	GGCTGGTCTC	CAACTCCTAA	TCTCAGGTGA	TCTACCCACC	TTGGCCTCCC	AAATTGCTGG	6336
	GATTACAGGC	GTGAACCACT	GCTCCCTTCC	CTGTCCTTCT	GATTTTAAAA	TAATAATACC	6396
20	AGCAGGAGGA	CGTCCAGACA	CAGCATAGGC	TACCTGCCAT	GCCCAACCGG	TGGGACATTT	6456
	GAGTTGCTTG	CTTGGCACTG	TCCTCTCATG	CGTTGGGTCC	ACTCAGTAGA	TGCCTGTTGA	6516
25	ATTCGTAATC	ATGGTCATAG	CTGTTTCCTG	TGTGAAATTG	TTATCCGCTC	ACAATTCAC	6576
	ACAACATACG	AGCCGGAAGC	ATAAAGTGTA	AAGCCTGGGG	TGCCTAATGA	GTGAGCTAAC	6636
	TCACATTAAT	TGCGTTGCGC	TCACTGCCCC	CTTTCAGTC	GGGAAACCTG	TCGTGCCAGC	6696
30	TGCATTAATG	AATCGGCCAA	CGCGCGGGGA	GAGGCGGTTT	GCGTATTGGG	CGCTCTTCCG	6756
	CTTCCTCGCT	CACTGACTCG	CTGCGCTCGG	TCGTTGCGCT	GCGGCGAGCG	GTATCAGCTC	6816
35	ACTCAAAGGC	GGTAATACGG	TTATCCACAG	AATCAGGGGA	TAACGCAGGA	AAGAATCATG	6876
	GAGCAAAAGG	CCAGCAAAAG	GCCAGGAACC	GTA AAAAGGC	CGCGTTGCTG	GCGTTTTTCC	6936
	ATAGGCTCCG	CCCCCTGAC	GAGCATCACA	AAAATCGACG	CTCAAGTCAG	AGGTGGCGAA	6996
40	ACCCGACAGG	ACTATAAAGA	TACCAGGCGT	TTCCCCCTGG	AAGCTCCCTC	GTGCGCTCTC	7056
	CTGTTCCGAC	CCTGCCGCTT	ACCGGATACC	TGTCCGCCTT	TCTCCCTTCG	GGAAGCGTGG	7116
45	CGCTTTCTCA	TAGCTCACGC	TGTAGGTATC	TCAGTTGCGT	GTAGGTCGTT	CGCTCCAAGC	7176
	TGGGCTGTGT	GCACGAACCC	CCCGTTCAGC	CCGACCGCTG	CGCCTTATCC	GGTAACTATC	7236
	GTCTTGAGTC	CAACCCGGTA	AGACACGACT	TATCGCCACT	GGCAGCAGCC	ACTGGTAACA	7296
50	GGATTAGCAG	AGCGAGGTAT	GTAGGCGGTG	CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT	7356
	ACGGCTACAC	TAGAAGGACA	GTATTTGGTA	TCTGCGCTCT	GCTGAAGCCA	GTTACCTTCG	7416
55	GAAAAAGAGT	TGGTAGCTCT	TGATCCGGCA	AACAAACCAC	CGCTGGTAGC	GGTGGTTTTT	7476

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	TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT CCTTTGATCT	7536
5	TTTCTACGGG GTCTGACGCT CAGTGGAACG AAAACTCAGC TTAAGGGATT TTGGTCATGA	7596
	GATTATCAAA AAGGATCTTC ACCTAGATCC TTTTAAATTA AAAATGAAGT TTTAAATCAA	7656
	TCTAAAGTAT ATATGAGTAA ACTTGGTCTG ACAGTTACCA ATGCTTAATC AGTGAGGCAC	7716
10	CTATCTCAGC GATCTGTCTA TTTCGTTTCAT CCATAGTTGC CTGACTCCCC GTCGTGTAGA	7776
	TAACTACGAT ACGGGAGGGC TTACCATCTG GCCCCAGTGC TGCAATGATA CCGCGAGACC	7836
	CACGCTCACC GGCTCCAGAT TTATCAGCAA TAAACCAGCC AGCCGGAAGG GCCGAGCGCA	7896
15	GAAGTGGTCC TGCAACTTTA TCCGCCTCCA TCCAGTCTAT TAATTGTTGC CGGGAAGCTA	7956
	GAGTAAGTAG TTCGCCAGTT AATAGTTTGC GCAACGTTGT TGCCATTGCT ACAGGCATCG	8016
20	TGGTGTACAG CTCGTCGTTT GGTATGGCTT CATTGAGCTC CGGTTCCCAA CGATCAAGGC	8076
	GAGTTACATG ATCCCCCATG TTGTGCAAAA AAGCGGTTAG CTCCTTCGGT CCTCCGATCG	8136
	TTGTCAGAAG TAAGTTGGCC GCAGTGTTAT CACTCATGGT TATGGCAGCA CTGCATAATT	8196
25	CTCTTACTGT CATGCCATCC GTAAGATGCT TTTCTGTGAC TGGTGAGTAC TCAACCAAGT	8256
	CATTCTGAGA ATAGTGATG CGGCGACCGA GTTGCTCTTG CCCGGCGTCA ATACGGGATA	8316
30	ATACCGCGCC ACATAGCAGA ACTTTAAAAG TGCTCATCAT TGGAACAGT TCTTCGGGGC	8376
	GAAAACTCTC AAGGATCTTA CCGCTGTTGA GATCCAGTTC GATGTAACCC ACTCGTCAC	8436
	CCAACTGATC TTCAGCATCT TTTACTTTCA CCAGCGTTTC TGGGTGAGCA AAAACAGGAA	8496
35	GGCAAAATGC CGCAAAAAAG GGAATAAGGG CGACACGGAA ATGTTGAATA CTCATACTCT	8556
	TCCTTTTTCA ATATTATTGA AGCATTTATC AGGGTTATTG TCTCATGAGC GGATACATAT	8616
40	TTGAATGTAT TTAGAAAAAT AAACAAATAG GGGTTCCGCG CACATTTCCT CGAAAAGTGC	8676
	CACCTGACGT CTAAGAAACC ATTATTATCA TGACATTAAC CTATAAAAAT AGGCGTATCA	8736
	CGAGGCCCTT TCGTCTCGCG CGTTTCGGTG ATGACGGTGA AAACCTCTGA CACATGCAGC	8796
45	TCCCGGAGAC GGTCACAGCT TGTCTGTAAG CGGATGCCGG GAGCAGACAA GCCCGTCAGG	8856
	GCGCGTCAGC GGGTGTGGC GGGTGTGGG GCTGGCTTAA CTATGCGGCA TCAGAGCAGA	8916
50	TTGTACTGAG AGTGCACCAT ATGCGGTGTG AAATACCGCA CAGATGCGTA AGGAGAAAAT	8976
	ACCGCATCAG GCGCCATTCT CCATTGAGGC TGCGCAACTG TTGGGAAGGG CGATCGGTGC	9036
55	GGGCCTCTTC GCTATTACGC CAGCTGGCGA AAGGGGGATG TGCTGCAAGG CGATTAAAGT	9096



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GGGTAACGCC AGGGTTTTC CAGTCACGAC GTTGTAAAC GACGGCCAGT GCCAAGCTTG 9156  
GGCTGCAG 9164

## 5 (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 12022 base pairs  
(B) TYPE: nucleic acid  
10 (C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## 15 (ix) FEATURE:

- (A) NAME/KEY: CDS  
(B) LOCATION: 1006..3294

## 20 (ix) FEATURE:

- (A) NAME/KEY: CDS  
(B) LOCATION: 6153..8234

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

25 GTCGACGGTA TCGATAAGCT TGATATCGAA TTCCTGCAGC CCGGGGGATC CACTAGTACT 60  
CGAGACCTAG GAGTTAATTT TTA AAAAGCA GTCAAAAGTC CAAGTGGCCC TTGCGAGCAT 120  
TTACTCTCTC TGTTTGCTCT GGTAAATAAT CTCAGGAGCA CAAACATTCC TTACTAGTCC 180  
30 TAGAAGTTAA TTTTAAAAA GCAGTCAAAA GTCCAAGTGG CCCTTGCGAG CATTTACTCT 240  
CTCTGTTTGC TCTGGTTAAT AATCTCAGGA GCACAAACAT TCCTTACTAG TTCTAGAGCG 300  
35 GCCGCCAGTG TGCTGGAATT CGGCTTTTTT AGGGCTGGAA GCTACCTTTG ACATCATTTT 360  
CTCTGCGAAT GCATGTATAA TTTCTACAGA ACCTATTAGA AAGGATCACC CAGCCTCTGC 420  
TTTTGTACAA CTTTCCCTTA AAAAAGTCC AATTCCACTG CTGTTTGGCC CAATAGTGAG 480  
40 AACTTTTTCC TGCTGCCTCT TGGTGCTTTT GCCTATGGCC CCTATTCTGC CTGCTGAAGA 540  
CACTCTTGCC AGCATGGACT TAAACCCCTC CAGCTCTGAC AATCCTCTTT CTCTTTTGTT 600  
45 TTACATGAAG GGTCTGGCAG CCAAAGCAAT CACTCAAAGT TCAAACCTTA TCATTTTTTG 660  
CTTTGTTTCCT CTTGGCCTTG GTTTGTACA TCAGCTTTGA AAATACCATC CCAGGGTTAA 720  
TGCTGGGGTT AATTTATAAC TAAGAGTGCT CTAGTTTTCG AATACAGGAC ATGCTATAAA 780  
50 AATGGAAGA TGTTGCTTTC TGAGAGATCT CGAGGAAGCT AACACAAAG AACACAAAC 840  
AACAAACAGG TAAGTATCCT TTTTACAGCA CAACTTAATG AGACAGATAG AAAGTGGTCT 900  
55 TGTAGAAACA GAGTAGTCGC CTGCTTTTCT GCCAGGTGCT GACTTCTCTC CCCTTCTCTT 960

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	TTTTCTTTT CTCAGGATAA CAAGAACGAA ACAATAACAG CCACC ATG GAA ATA	1014
	Met Glu Ile	
	1	
5	GAG CTC TCC ACC TGC TTC TTT CTG TGC CTT TTG CGA TTC TGC TTT AGT	1062
	Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe Cys Phe Ser	
	5 10 15	
10	GCC ACC AGA AGA TAC TAC CTG GGT GCA GTG GAA CTG TCA TGG GAC TAT	1110
	Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser Trp Asp Tyr	
	20 25 30 35	
15	ATG CAA AGT GAT CTC GGT GAG CTG CCT GTG GAC GCA AGA TTT CCT CCT	1158
	Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg Phe Pro Pro	
	40 45 50	
20	AGA GTG CCA AAA TCT TTT CCA TTC AAC ACC TCA GTC GTG TAC AAA AAG	1206
	Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val Tyr Lys Lys	
	55 60 65	
25	ACT CTG TTT GTA GAA TTC ACG GTT CAC CTT TTC AAC ATC GCT AAG CCA	1254
	Thr Leu Phe Val Glu Phe Thr Val His Leu Phe Asn Ile Ala Lys Pro	
	70 75 80	
30	AGG CCA CCC TGG ATG GGT CTG CTA GGT CCT ACC ATC CAG GCT GAG GTT	1302
	Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln Ala Glu Val	
	85 90 95	
35	TAT GAT ACA GTG GTC ATT ACA CTT AAG AAC ATG GCT TCC CAT CCT GTC	1350
	Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser His Pro Val	
	100 105 110 115	
40	AGT CTT CAT GCT GTT GGT GTA TCC TAC TGG AAA GCT TCT GAG GGA GCT	1398
	Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser Glu Gly Ala	
	120 125 130	
45	GAA TAT GAT GAT CAG ACC AGT CAA AGG GAG AAA GAA GAT GAT AAA GTC	1446
	Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp Asp Lys Val	
	135 140 145	
50	TTC CCT GGT GGA AGC CAT ACA TAT GTC TGG CAG GTC CTG AAA GAG AAT	1494
	Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu Lys Glu Asn	
	150 155 160	
55	GGT CCA ATG GCC TCT GAC CCA CTG TGC CTT ACC TAC TCA TAT CTT TCT	1542
	Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser Tyr Leu Ser	
	165 170 175	
60	CAT GTG GAC CTG GTA AAA GAC TTG AAT TCA GGC CTC ATT GGA GCC CTA	1590
	His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile Gly Ala Leu	
	180 185 190 195	

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	CTA GTA TGT AGA GAA GGG AGT CTG GCC AAG GAA AAG ACA CAG ACC TTG	1638
	Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr Gln Thr Leu	
	200 205 210	
5	CAC AAA TTT ATA CTA CTT TTT GCT GTA TTT GAT GAA GGG AAA AGT TGG	1686
	His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly Lys Ser Trp	
	215 220 225	
10	CAC TCA GAA ACA AAG AAC TCC TTG ATG CAG GAT AGG GAT GCT GCA TCT	1734
	His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp Ala Ala Ser	
	230 235 240	
15	GCT CGG GCC TGG CCT AAA ATG CAC ACA GTC AAT GGT TAT GTA AAC AGG	1782
	Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr Val Asn Arg	
	245 250 255	
20	TCT CTG CCA GGT CTG ATT GGA TGC CAC AGG AAA TCA GTC TAT TGG CAT	1830
	Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val Tyr Trp His	
	260 265 270 275	
25	GTG ATT GGA ATG GGC ACC ACT CCT GAA GTG CAC TCA ATA TTC CTC GAA	1878
	Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile Phe Leu Glu	
	280 285 290	
30	GGT CAC ACA TTT CTT GTG AGG AAC CAT CGC CAG GCG TCC TTG GAA ATC	1926
	Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser Leu Glu Ile	
	295 300 305	
35	TCG CCA ATA ACT TTC CTT ACT GCT CAA ACA CTC TTG ATG GAC CTT GGA	1974
	Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met Asp Leu Gly	
	310 315 320	
40	CAG TTT CTA CTG TTT TGT CAT ATC TCT TCC CAC CAA CAT GAT GGC ATG	2022
	Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His Asp Gly Met	
	325 330 335	
45	GAA GCT TAT GTC AAA GTA GAC AGC TGT CCA GAG GAA CCC CAA CTA CGA	2070
	Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro Gln Leu Arg	
	340 345 350 355	
50	ATG AAA AAT AAT GAA GAA GCG GAA GAC TAT GAT GAT GAT CTT ACT GAT	2118
	Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp Leu Thr Asp	
	360 365 370	
55	TCT GAA ATG GAT GTG GTC AGG TTT GAT GAT GAC AAC TCT CCT TCC TTT	2166
	Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser Pro Ser Phe	
	375 380 385	
60	ATC CAA ATT CGC TCA GTT GCC AAG AAG CAT CCT AAA ACT TGG GTA CAT	2214
	Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr Trp Val His	
	390 395 400	
65	TAC ATT GCT GCT GAA GAG GAG GAC TGG GAC TAT GCT CCC TTA GTC CTC	2262
	Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro Leu Val Leu	
	405 410 415	

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	GCC CCC GAT GAC AGA AGT TAT AAA AGT CAA TAT TTG AAC AAT GGC CCT	2310
	Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn Asn Gly Pro	
	420 425 430 435	
5	CAG CGG ATT GGT AGG AAG TAC AAA AAA GTC CGA TTT ATG GCA TAC ACA	2358
	Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met Ala Tyr Thr	
	440 445 450	
10	GAT GAA ACC TTT AAG ACT CGT GAA GCT ATT CAG CAT GAA TCA GGA ATC	2406
	Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu Ser Gly Ile	
	455 460 465	
	TTG GGA CCT TTA CTT TAT GGG GAA GTT GGA GAC ACA CTG TTG ATT ATA	2454
15	Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu Leu Ile Ile	
	470 475 480	
	TTT AAG AAT CAA GCA AGC AGA CCA TAT AAC ATC TAC CCT CAC GGA ATC	2502
20	Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro His Gly Ile	
	485 490 495	
	ACT GAT GTC CGT CCT TTG TAT TCA AGG AGA TTA CCA AAA GGT GTA AAA	2550
25	Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys Gly Val Lys	
	500 505 510 515	
	CAT TTG AAG GAT TTT CCA ATT CTG CCA GGA GAA ATA TTC AAA TAT AAA	2598
	His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe Lys Tyr Lys	
	520 525 530	
30	TGG ACA GTG ACT GTA GAA GAT GGG CCA ACT AAA TCA GAT CCT CGG TGC	2646
	Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp Pro Arg Cys	
	535 540 545	
	CTG ACC CGC TAT TAC TCT AGT TTC GTT AAT ATG GAG AGA GAT CTA GCT	2694
35	Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg Asp Leu Ala	
	550 555 560	
	TCA GGA CTC ATT GGC CCT CTC CTC ATC TGC TAC AAA GAA TCT GTA GAT	2742
40	Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu Ser Val Asp	
	565 570 575	
	CAA AGA GGA AAC CAG ATA ATG TCA GAC AAG AGG AAT GTC ATC CTG TTT	2790
45	Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val Ile Leu Phe	
	580 585 590 595	
	TCT GTA TTT GAT GAG AAC CGA AGC TGG TAC CTC ACA GAG AAT ATA CAA	2838
	Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu Asn Ile Gln	
	600 605 610	
50	CGC TTT CTC CCC AAT CCA GCT GGA GTG CAG CTT GAG GAT CCA GAG TTC	2886
	Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp Pro Glu Phe	
	615 620 625	

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	CAA GCC TCC AAC ATC ATG CAC AGC ATC AAT GGC TAT GTT TTT GAT AGT	2934
	Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val Phe Asp Ser	
	630 635 640	
5	TTG CAG TTG TCA GTT TGT TTG CAT GAG GTG GCA TAC TGG TAC ATT CTA	2982
	Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp Tyr Ile Leu	
	645 650 655	
10	AGC ATT GGA GCA CAG ACT GAC TTC CTT TCT GTC TTC TTC TCT GGA TAT	3030
	Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe Ser Gly Tyr	
	660 665 670 675	
15	ACC TTC AAA CAC AAA ATG GTC TAT GAA GAC ACA CTC ACC CTA TTC CCA	3078
	Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr Leu Phe Pro	
	680 685 690	
20	TTC TCA GGA GAA ACT GTC TTC ATG TCG ATG GAA AAC CCA GGT CTA TGG	3126
	Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro Gly Leu Trp	
	695 700 705	
25	ATT CTG GGG TGC CAC AAC TCA GAC TTT CGG AAC AGA GGC ATG ACC GCC	3174
	Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly Met Thr Ala	
	710 715 720	
30	TTA CTG AAG GTT TCT AGT TGT GAC AAG AAC ACT GGT GAT TAT TAC GAG	3222
	Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp Tyr Tyr Glu	
	725 730 735	
35	GAC AGT TAT GAA GAT ATT TCA GCA TAC TTG CTG AGT AAA AAC AAT GCC	3270
	Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys Asn Asn Ala	
	740 745 750 755	
40	ATT GAA CCA AGA AGC TTC TCC CAG GTAAGTTATT ATATAAATTC AAGACACCCCT	3324
	Ile Glu Pro Arg Ser Phe Ser Gln	
	760	
45	AGCACTAGGC AAAAGCAATT TAATGCCACC ACAATTCCAG AAAATGACAT AGAGAAGACT	3384
	GACCCCTGGT TTGCACACAG AACACCTATG CCTAAAATAC AAAATGTCTC CTCTAGTGAT	3444
	TTGTTGATGC TCTTGCGACA GAGTCCTACT CCACATGGGC TATCCTTATC TGATCTCCAA	3504
	GAAGCCAAAT ATGAGACTTT TTCTGATGAT CCATCACCTG GAGCAATAGA CAGTAATAAC	3564
50	AGCCTGTCTG AAATGACACA CTTCAGGCCA CAGCTCCATC ACAGTGGGGA CATGGTATTT	3624
	ACCCCTGAGT CAGGCCTCCA ATTAAGATTA AATGAGAAAC TGGGGACAAC TGCAGCAACA	3684
	GAGTTGAAGA AACTTGATTT CAAAGTTTCT AGTACATCAA ATAATCTGAT TTCAACAATT	3744
	CCATCAGACA ATTTGGCAGC AGGTACTGAT AATACAAGTT CCTTAGGACC CCCAAGTATG	3804
	CCAGTTCATT ATGATAGTCA ATTAGATACC ACTCTATTTG GCAAAAAGTC ATCTCCCTT	3864
55	ACTGAGTCTG GTGGACCTCT GAGCTTGAGT GAAGAAAATA ATGATTCAAA GTTGTTAGAA	3924

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TCAGGTTTAA TGAATAGCCA AGAAAGTTCA TGGGGAAAAA ATGTATCGTC AACAGAGAGT 3984  
 GGTAGGTTAT TTAAAGGGAA AAGAGCTCAT GGACCTGCTT TGTTGACTAA AGATAATGCC 4044  
 5 TTATTCAAAG TTAGCATCTC TTTGTTAAAG ACAAACAAAA CTTCCAATAA TTCAGCAACT 4104  
 AATAGAAAGA CTCACATTGA TGGCCCATCA TTATTAATTG AGAATAGTCC ATCAGTCTGG 4164  
 10 CAAAATATAT TAGAAAGTGA CACTGAGTTT AAAAAAGTGA CACCTTTGAT TCATGACAGA 4224  
 ATGCTTATGG ACAAAAATGC TACAGCTTTG AGGCTAAATC ATATGTCAA TAAAACTACT 4284  
 TCATCAAAAA ACATGGAAAT GGTCCAACAG AAAAAAGAGG GCCCCATTCC ACCAGATGCA 4344  
 15 CAAAATCCAG ATATGTCGTT CTTTAAGATG CTATTCTTGC CAGAATCAGC AAGGTGGATA 4404  
 CAAAGGACTC ATGGAAAGAA CTCTCTGAAC TCTGGGCAAG GCCCCAGTCC AAAGCAATTA 4464  
 20 GTATCCTTAG GACCAGAAAA ATCTGTGGAA GGTGAGAATT TCTTGTCTGA GAAAAACAAA 4524  
 GTGGTAGTAG GAAAGGGTGA ATTTACAAAG GACGTAGGAC TCAAAGAGAT GGTTTTTCCA 4584  
 AGCAGCAGAA ACCTATTTCT TACTAACTTG GATAATTAC ATGAAAATAA TACACACAAT 4644  
 25 CAAGAAAAAA AAATTCAGGA AGAAATAGAA AAGAAGGAAA CATTAATCCA AGAGAATGTA 4704  
 GTTTTGCCCTC AGATACATAC AGTGACTGGC ACTAAGAATT TCATGAAGAA CCTTTTCTTA 4764  
 30 CTGAGCACTA GGCAAAATGT AGAAGGTTCA TATGAGGGGG CATATGCTCC AGTACTTCAA 4824  
 GATTTTAGGT CATTAATGA TTCAACAAAT AGAACAAAGA AACACACAGC TCATTTCTCA 4884  
 AAAAAAGGGG AGGAAGAAAA CTTGGAAGGC TTGGGAAATC AAACCAAGCA AATTGTAGAG 4944  
 35 AAATATGCAT GCACCACAAG GATATCTCCT AATACAAGCC AGCAGAATTT TGTCACGCAA 5004  
 CGTAGTAAGA GAGCTTTGAA ACAATTCAGA CTCCCCTAG AAGAAACAGA ACTTGAAAAA 5064  
 40 AGGATAATTG TGGATGACAC CTCAACCCAG TGGTCCAAAA ACATGAAACA TTTGACCCCG 5124  
 AGCACCTCA CACAGATAGA CTACAATGAG AAGGAGAAAG GGGCCATTAC TCAGTCTCCC 5184  
 TTATCAGATT GCCTTACGAG GAGTCATAGC ATCCCTCAAG CAAATAGATC TCCATTACCC 5244  
 45 ATTGCAAAGG TATCATCATT TCCATCTATT AGACCTATAT ATCTGACCAG GGTCCATATC 5304  
 CAAGACAACT CTCTCATCT TCCAGCAGCA TCTTATAGAA AGAAAGATTG TGGGGTCCAA 5364  
 50 GAAAGCAGTC ATTTCTTACA AGGAGCCAAA AAAAATAACC TTTCTTTAGC CATTCTAACC 5424  
 TTGGAGATGA CTGGTGATCA AAGAGAGGTT GGCTCCCTGG GGACAAGTGC CACAAATTCA 5484  
 GTCACATACA AGAAAGTTGA GAACACTGTT CTCCCGAAAC CAGACTTGCC CAAAACATCT 5544  
 55

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	GGCAAAGTTG AATTGCTTCC AAAAGTTCAC ATTTATCAGA AGGACCTATT CCCTACGGAA	5604
	ACTAGCAATG GGTCTCCTGG CCATCTGGAT CTCGTGGAAG GGAGCCTTCT TCAGGGAACA	5664
5	GAGGGAGCGA TTAAGTGGAA TGAAGCAAAC AGACCTGGAA AAGTTCCCTT TCTGAGAGTA	5724
	GCAACAGAAA GCTCTGCAAA GACTCCCTCC AAGCTATTGG ATCCTCTTGC TTGGGATAAC	5784
10	CACTATGGTA CTCAGATACC AAAAGAAGAG TGGAAATCCC AAGAGAAGTC ACCAGAAAAA	5844
	ACAGCTTTTA AGAAAAAGGA TACCATTTTG TCCCTGAACG CTTGTGAAAG CAATCATGCA	5904
	ATAGCAGCAA TAAATGAGGG ACAAATAAG CCCGAAATAG AAGTCACCTG GGCAAAGCAA	5964
15	GGTAGGACTG AAAGGCTGTG CTCTCAATTG TGCTAATAAA GCTTGGCAAG AGTATTTCAA	6024
	GGAAGATGAA GTCATTA ACT ATGCAAAATG CTTCTCAGGC ACCTAGGAAA ATGAGGATGT	6084
20	GAGGCATTTC TACCCACTTG GTACATAAAA TTATTGGGTC ACCCTTTTCC TCTTCTTTTT	6144
	TTCTCCAG AAC CCA CCA GTC TTG AAA CGC CAT CAA CGG GAA ATA ACT CGT	6194
	Asn Pro Pro Val Leu Lys Arg His Gln Arg Glu Ile Thr Arg	
	1 5 10	
25	ACT ACT CTT CAG TCA GAT CAA GAG GAA ATT GAC TAT GAT GAT ACC ATA	6242
	Thr Thr Leu Gln Ser Asp Gln Glu Glu Ile Asp Tyr Asp Asp Thr Ile	
	15 20 25 30	
30	TCA GTT GAA ATG AAG AAG GAA GAT TTT GAC ATT TAT GAT GAG GAT GAA	6290
	Ser Val Glu Met Lys Lys Glu Asp Phe Asp Ile Tyr Asp Glu Asp Glu	
	35 40 45	
35	AAT CAG AGC CCC CGC AGC TTT CAA AAG AAA ACA CGA CAC TAT TTT ATT	6338
	Asn Gln Ser Pro Arg Ser Phe Gln Lys Lys Thr Arg His Tyr Phe Ile	
	50 55 60	
40	GCT GCA GTG GAG AGG CTC TGG GAT TAT GGG ATG AGT AGC TCC CCA CAT	6386
	Ala Ala Val Glu Arg Leu Trp Asp Tyr Gly Met Ser Ser Ser Pro His	
	65 70 75	
45	GTT CTA AGA AAC AGG GCT CAG AGT GGC AGT GTC CCT CAG TTC AAG AAA	6434
	Val Leu Arg Asn Arg Ala Gln Ser Gly Ser Val Pro Gln Phe Lys Lys	
	80 85 90	
50	GTT GTT TTC CAG GAA TTT ACT GAT GGC TCC TTT ACT CAG CCC TTA TAC	6482
	Val Val Phe Gln Glu Phe Thr Asp Gly Ser Phe Thr Gln Pro Leu Tyr	
	95 100 105 110	
55	CGT GGA GAA CTA AAT GAA CAT TTG GGA CTC CTG GGG CCA TAT ATA AGA	6530
	Arg Gly Glu Leu Asn Glu His Leu Gly Leu Leu Gly Pro Tyr Ile Arg	
	115 120 125	
55	GCA GAA GTT GAA GAT AAT ATC ATG GTA ACT TTC AGA AAT CAG GCC TCT	6578
	Ala Glu Val Glu Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser	
	130 135 140	

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	CGT CCC TAT TCC TTC TAT TCT AGC CTT ATT TCT TAT GAG GAA GAT CAG	6626
	Arg Pro Tyr Ser Phe Tyr Ser Ser Leu Ile Ser Tyr Glu Glu Asp Gln	
	145 150 155	
5	AGG CAA GGA GCA GAA CCT AGA AAA AAC TTT GTC AAG CCT AAT GAA ACC	6674
	Arg Gln Gly Ala Glu Pro Arg Lys Asn Phe Val Lys Pro Asn Glu Thr	
	160 165 170	
10	AAA ACT TAC TTT TGG AAA GTG CAA CAT CAT ATG GCA CCC ACT AAA GAT	6722
	Lys Thr Tyr Phe Trp Lys Val Gln His His Met Ala Pro Thr Lys Asp	
	175 180 185 190	
15	GAG TTT GAC TGC AAA GCC TGG GCT TAT TTC TCT GAT GTT GAC CTG GAA	6770
	Glu Phe Asp Cys Lys Ala Trp Ala Tyr Phe Ser Asp Val Asp Leu Glu	
	195 200 205	
20	AAA GAT GTG CAC TCA GGC CTG ATT GGA CCC CTT CTG GTC TGC CAC ACT	6818
	Lys Asp Val His Ser Gly Leu Ile Gly Pro Leu Leu Val Cys His Thr	
	210 215 220	
25	AAC ACA CTG AAC CCT GCT CAT GGG AGA CAA GTG ACA GTA CAG GAA TTT	6866
	Asn Thr Leu Asn Pro Ala His Gly Arg Gln Val Thr Val Gln Glu Phe	
	225 230 235	
30	GCT CTG TTT TTC ACC ATC TTT GAT GAG ACC AAA AGC TGG TAC TTC ACT	6914
	Ala Leu Phe Phe Thr Ile Phe Asp Glu Thr Lys Ser Trp Tyr Phe Thr	
	240 245 250	
35	GAA AAT ATG GAA AGA AAC TGC AGG GCT CCC TGC AAT ATC CAG ATG GAA	6962
	Glu Asn Met Glu Arg Asn Cys Arg Ala Pro Cys Asn Ile Gln Met Glu	
	255 260 265 270	
40	GAT CCC ACT TTT AAA GAG AAT TAT CGC TTC CAT GCA ATC AAT GGC TAC	7010
	Asp Pro Thr Phe Lys Glu Asn Tyr Arg Phe His Ala Ile Asn Gly Tyr	
	275 280 285	
45	ATA ATG GAT ACA CTA CCT GGC TTA GTA ATG GCT CAG GAT CAA AGG ATT	7058
	Ile Met Asp Thr Leu Pro Gly Leu Val Met Ala Gln Asp Gln Arg Ile	
	290 295 300	
50	CGA TGG TAT CTG CTC AGC ATG GGC AGC AAT GAA AAC ATC CAT TCT ATT	7106
	Arg Trp Tyr Leu Leu Ser Met Gly Ser Asn Glu Asn Ile His Ser Ile	
	305 310 315	
55	CAT TTC AGT GGA CAT GTG TTC ACT GTA CGA AAA AAA GAG GAG TAT AAA	7154
	His Phe Ser Gly His Val Phe Thr Val Arg Lys Lys Glu Glu Tyr Lys	
	320 325 330	
60	ATG GCA CTG TAC AAT CTC TAT CCA GGT GTT TTT GAG ACA GTG GAA ATG	7202
	Met Ala Leu Tyr Asn Leu Tyr Pro Gly Val Phe Glu Thr Val Glu Met	
	335 340 345 350	



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	TTA CCA TCC AAA GCT GGA ATT TGG CGG GTG GAA TGC CTT ATT GGC GAG	7250
	Leu Pro Ser Lys Ala Gly Ile Trp Arg Val Glu Cys Leu Ile Gly Glu	
	355 360 365	
5	CAT CTA CAT GCT GGG ATG AGC ACA CTT TTT CTG GTG TAC AGC AAT AAG	7298
	His Leu His Ala Gly Met Ser Thr Leu Phe Leu Val Tyr Ser Asn Lys	
	370 375 380	
10	TGT CAG ACT CCC CTG GGA ATG GCT TCT GGA CAC ATT AGA GAT TTT CAG	7346
	Cys Gln Thr Pro Leu Gly Met Ala Ser Gly His Ile Arg Asp Phe Gln	
	385 390 395	
15	ATT ACA GCT TCA GGA CAA TAT GGA CAG TGG GCC CCA AAG CTG GCC AGA	7394
	Ile Thr Ala Ser Gly Gln Tyr Gly Gln Trp Ala Pro Lys Leu Ala Arg	
	400 405 410	
20	CTT CAT TAT TCC GGA TCA ATC AAT GCC TGG AGC ACC AAG GAG CCC TTT	7442
	Leu His Tyr Ser Gly Ser Ile Asn Ala Trp Ser Thr Lys Glu Pro Phe	
	415 420 425 430	
25	TCT TGG ATC AAG GTG GAT CTG TTG GCA CCA ATG ATT ATT CAC GGC ATC	7490
	Ser Trp Ile Lys Val Asp Leu Leu Ala Pro Met Ile Ile His Gly Ile	
	435 440 445	
30	AAG ACC CAG GGT GCC CGT CAG AAG TTC TCC AGC CTC TAC ATC TCT CAG	7538
	Lys Thr Gln Gly Ala Arg Gln Lys Phe Ser Ser Leu Tyr Ile Ser Gln	
	450 455 460	
35	TTT ATC ATC ATG TAT AGT CTT GAT GGG AAG AAG TGG CAG ACT TAT CGA	7586
	Phe Ile Ile Met Tyr Ser Leu Asp Gly Lys Lys Trp Gln Thr Tyr Arg	
	465 470 475	
40	GGA AAT TCC ACT GGA ACC TTA ATG GTC TTC TTT GGC AAT GTG GAT TCA	7634
	Gly Asn Ser Thr Gly Thr Leu Met Val Phe Phe Gly Asn Val Asp Ser	
	480 485 490	
45	TCT GGG ATA AAA CAC AAT ATT TTT AAC CCT CCA ATT ATT GCT CGA TAC	7682
	Ser Gly Ile Lys His Asn Ile Phe Asn Pro Pro Ile Ile Ala Arg Tyr	
	495 500 505 510	
50	ATC CGT TTG CAC CCA ACT CAT TAT AGC ATT CGC AGC ACT CTT CGC ATG	7730
	Ile Arg Leu His Pro Thr His Tyr Ser Ile Arg Ser Thr Leu Arg Met	
	515 520 525	
55	GAG TTG ATG GGC TGT GAT TTA AAT AGT TGC AGC ATG CCA TTG GGA ATG	7778
	Glu Leu Met Gly Cys Asp Leu Asn Ser Cys Ser Met Pro Leu Gly Met	
	530 535 540	
60	GAG AGT AAA GCA ATA TCA GAT GCA CAG ATT ACT GCT TCA TCC TAC TTT	7826
	Glu Ser Lys Ala Ile Ser Asp Ala Gln Ile Thr Ala Ser Ser Tyr Phe	
	545 550 555	
65	ACC AAT ATG TTT GCC ACC TGG TCT CCT TCA AAA GCT CGA CTT CAC CTC	7874
	Thr Asn Met Phe Ala Thr Trp Ser Pro Ser Lys Ala Arg Leu His Leu	
	560 565 570	

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5	CAA GGG AGG AGT AAT GCC TGG AGA CCT CAG GTG AAT AAT CCA AAA GAG	7922
	Gln Gly Arg Ser Asn Ala Trp Arg Pro Gln Val Asn Asn Pro Lys Glu	
	575 580 585 590	
10	TGG CTG CAA GTG GAC TTC CAG AAG ACA ATG AAA GTC ACA GGA GTA ACT	7970
	Trp Leu Gln Val Asp Phe Gln Lys Thr Met Lys Val Thr Gly Val Thr	
	595 600 605	
15	ACT CAG GGA GTA AAA TCT CTG CTT ACC AGC ATG TAT GTG AAG GAG TTC	8018
	Thr Gln Gly Val Lys Ser Leu Leu Thr Ser Met Tyr Val Lys Glu Phe	
	610 615 620	
20	CTC ATC TCC AGC AGT CAA GAT GGC CAT CAG TGG ACT CTC TTT TTT CAG	8066
	Leu Ile Ser Ser Ser Gln Asp Gly His Gln Trp Thr Leu Phe Phe Gln	
	625 630 635	
25	AAT GGC AAA GTA AAG GTT TTT CAG GGA AAT CAA GAC TCC TTC ACA CCT	8114
	Asn Gly Lys Val Lys Val Phe Gln Gly Asn Gln Asp Ser Phe Thr Pro	
	640 645 650	
30	GTG GTG AAC TCT CTA GAC CCA CCG TTA CTG ACT CGC TAC CTT CGA ATT	8162
	Val Val Asn Ser Leu Asp Pro Pro Leu Leu Thr Arg Tyr Leu Arg Ile	
	655 660 665 670	
35	CAC CCC CAG AGT TGG GTG CAC CAG ATT GCC CTG AGG ATG GAG GTT CTG	8210
	His Pro Gln Ser Trp Val His Gln Ile Ala Leu Arg Met Glu Val Leu	
	675 680 685	
40	GGC TGC GAG GCA CAG GAC CTC TAC TGAGGGTGGC CACTGCAGCA CCTGCCACTG	8264
	Gly Cys Glu Ala Gln Asp Leu Tyr	
	690	
45	CCGTCACCTC TCCCTCCTCA GCTCCAGGGC AGTGTCCCTC CCTGGCTTGC CTTCTACCTT	8324
	TGTGCTAAAT CCTAGCAGAC ACTGCCTTGA AGCCTCCTGA ATTAACATATC ATCAGTCTCTG	8384
	CATTTCTTTG GTGGGGGGCC AGGAGGGTGC ATCCAATTTA ACTTAACTCT TACCTATTTT	8444
50	CTGCAGCTGC TCCCAGATTA CTCCTTCCTT CCAATATAAC TAGGCAAAAA GAAGTGAGGA	8504
	GAAACCTGCA TGAAAGCATT CTTCCCTGAA AAGTTAGGCC TCTCAGAGTC ACCACTTCCT	8564
55	CTGTTGTAGA AAAACTATGT GATGAAACTT TGAAAAAGAT ATTTATGATG TTAACCTGTT	8624
	TATTGCAGCT TATAATGGTT ACAAATAAAG CAATAGCATC ACAAATTTCA CAAATAAAGC	8684
	ATTTTTTTCA CTGCAATCTA GTTGTGGTTT GTCCAAACTC ATCAATGTAT CTTATCATGT	8744
60	CTGGATCCCC GGGTGGCATC CCTGTGACCC CTCCCCAGTG CCTCTCCTGG CCCTGGAAGT	8804
	TGCCACTCCA GTGCCCACCA GCCTTGTCTT AATAAAATTA AGTTGCATCA TTTTGTCTGA	8864
	CTAGGTGTCC TTCTATAATA TTATGGGGTG GAGGGGGGTG GTATGGAGCA AGGGGCAAGT	8924

TGGGAAGACA ACCTGTAGGG CCTGCGGGGT CTATTCGGGA ACCAAGCTGG AGTGCAGTGG 8984  
CACAATCTTG GCTCACTGCA ATCTCCGCCT CCTGGGTTC ACGATTCTC CTGCCTCAGC 9044  
5 CTCCCAGATT GTTGGGATTC CAGGCATGCA TGACCAGGCT CAGCTAATTT TTGTTTTTTT 9104  
GGTAGAGACG GGGTTTCACC ATATTGGCCA GGCTGGTCTC CAACTCCTAA TCTCAGGTGA 9164  
10 TCTACCCACC TTGGCCTCCC AAATTGCTGG GATTACAGGC GTGAACCACT GCTCCCTTCC 9224  
CTGTCTTCT GATTTTAAAA TAACTATACC AGCAGGAGGA CGTCCAGACA CAGCATAGGC 9284  
TACCTGCCAT GCCCAACCGG TGGGACATTT GAGTTGCTTG CTTGGCACTG TCCTCTCATG 9344  
15 CGTTGGGTCC ACTCAGTAGA TGCCTGTTGA ATTCTGAATC ATGGTCATAG CTGTTTCTTG 9404  
TGTGAAATG TTATCCGCTC ACAATTCCAC ACAACATACG AGCCGGAAGC ATAAAGTGTA 9464  
AAGCCTGGGG TGCCTAATGA GTGAGCTAAC TCACATTAAT TGCATTGCGC TCACTGCCCCG 9524  
20 CTTTCCAGTC GGGAAACCTG TCGTGCCAGC TGCATTAATG AATCGGCCAA CGCGCGGGGA 9584  
GAGGCGGTTT GCGTATTGGG CGCTCTTCCG CTTCTCGCT CACTGACTCG CTGCGCTCGG 9644  
25 TCGTTGCGCT GCGGCGAGCG GTATCAGCTC ACTCAAAGGC GGTAATACGG TTATCCACAG 9704  
AATCAGGGGA TAACGCAGGA AAGAACATGT GAGCAAAAGG CCAGCAAAAG GCCAGGAACC 9764  
GTAAAAAGGC CGCGTTGCTG GCGTTTTTCC ATAGGCTCCG CCCCCCTGAC GAGCATCACA 9824  
30 AAAATCGACG CTCAAGTCAG AGGTGGCGAA ACCCGACAGG ACTATAAAGA TACCAGGCGT 9884  
TTCCCCCTGG AAGCTCCCTC GTGCGCTCTC CTGTTCCGAC CTTGCCGCTT ACCGGATACC 9944  
35 TGTCCGCCTT TCTCCCTTCG GGAAGCGTGG CGCTTTCTCA TAGCTCACGC TGTAGGTATC 10004  
TCAGTTCCGT GTAGTCGTT CGCTCCAAGC TGGGCTGTGT GCACGAACCC CCCGTTACG 10064  
CCGACCGCTG CGCCTTATCC GGTAACTATC GTCTTGAGTC CAACCCGGTA AGACACGACT 10124  
40 TATCGCCACT GGCAGCAGCC ACTGGTAACA GGATTAGCAG AGCGAGGTAT GTAGGCGGTG 10184  
CTACAGAGTT CTTGAAGTGG TGGCCTAACT ACGGCTACAC TAGAAGGACA GTATTTGGTA 10244  
45 TCTGCGCTCT GCTGAAGCCA GTTACCTTCG GAAAAAGAGT TGGTAGCTCT TGATCCGCA 10304  
AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA 10364  
AAAAAGGATC TCAAGAAGAT CCTTTGATCT TTTCTACGGG GTCTGACGCT CAGTGGAACG 10424  
50 AAAACTCAG TTAAGGGATT TTGGTCATGA GATTATCAAA AAGGATCTTC ACCTAGATCC 10484  
TTTTAAATTA AAAATGAAGT TTTAAATCAA TCTAAAGTAT ATATGAGTAA ACTTGGTCTG 10544  
55 ACAGTTACCA ATGCTTAATC AGTGAGGCAC CTATCTCAGC GATCTGTCTA TTTCGTTTCA 10604

CCATAGTTGC CTGACTCCCC GTCGTGTAGA TAACTACGAT ACGGGAGGGC TTACCATCTG 10664  
5 GCCCCAGTGC TGCAATGATA CCGCGAGACC CACGCTCACC GGCTCCAGAT TTATCAGCAA 10724  
TAAACCAGCC AGCCGGAAGG GCCGAGCGCA GAAGTGGTCC TGCAACTTTA TCCGCCTCCA 10784  
TCCAGTCTAT TAATTGTTGC CGGGAAGCTA GAGTAAGTAG TTCGCCAGTT AATAGTTTGC 10844  
10 GCAACGTTGT TGCCATTGCT ACAGGCATCG TGGTGTACAG CTCGTCGTTT GGTATGGCTT 10904  
CATTACAGCTC CGGTTCCCAA CGATCAAGGC GAGTTACATG ATCCCCCATG TTGTGCAAAA 10964  
AAGCGGTTAG CTCCTTCGGT CCTCCGATCG TTGTCAGAAG TAAGTTGGCC GCAGTGTTAT 11024  
15 CACTCATGGT TATGGCAGCA CTGCATAATT CTCTTACTGT CATGCCATCC GTAAGATGCT 11084  
TTTCTGTGAC TGGTGAGTAC TCAACCAAGT CATCTGAGA ATAGTGTATG CGGCGACCGA 11144  
20 GTTGCTCTTG CCCGGCGTCA ATACGGGATA ATACCGCGCC ACATAGCAGA ACTTTAAAAG 11204  
TGCTCATCAT TGGAAAACGT TCTTCGGGGC GAAAACTCTC AAGGATCTTA CCGCTGTTGA 11264  
GATCCAGTTC GATGTAACCC ACTCGTGAC CCAACTGATC TTCAGCATCT TTTACTTTCA 11324  
25 CCAGCGTTTC TGGGTGAGCA AAAACAGGAA GGCAAAATGC CGCAAAAAG GGAATAAGGG 11384  
CGACACGGAA ATGTTGAATA CTCATACTCT TCCTTTTTC AATTATTGA AGCATTATC 11444  
30 AGGGTTATTG TCTCATGAGC GGATACATAT TTGAATGTAT TTAGAAAAAT AAACAAATAG 11504  
GGGTTCCGCG CACATTTCCC CGAAAAGTGC CACCTGACGT CTAAGAAACC ATTATTATCA 11564  
TGACATTAACTATAAAAAAT AGGCGTATCA CGAGGCCCTT TCGTCTCGCG CGTTTCGGTG 11624  
35 ATGACGGTGA AAACCTCTGA CACATGCAGC TCCCGGAGAC GGTCACAGCT TGTCTGTAAG 11684  
CGGATGCCGG GAGCAGACAA GCCCGTCAGG GCGCGTCAGC GGGTGTGGC GGGTGTGGG 11744  
40 GCTGGCTTAA CTATGCGGCA TCAGAGCAGA TTGTAAGTAT AGTGACCAT ATGCGGTGTG 11804  
AAATACCGCA CAGATGCGTA AGGAGAAAAT ACCGCATCAG GCGCCATTCT CCATTCAGGC 11864  
TGCGCAACTG TTGGGAAGGG CGATCGGTGC GGGCTCTTTC GCTATTACGC CAGCTGGCGA 11924  
45 AAGGGGGATG TGCTGCAAGG CGATTAAAGT GGGTAACGCC AGGGTTTTCC CAGTCACGAC 11984  
GTTGTAAAAC GACGGCCAGT GCCAAGCTTG GGCTGCAG 12022

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## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 11846 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

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## (ix) FEATURE:

- (A) NAME/KEY: CDS  
(B) LOCATION: 1006..8058

## 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GTCGACGGTA TCGATAAGCT TGATATCGAA TTCCTGCAGC CCGGGGGATC CACTAGTACT 60  
CGAGACCTAG GAGTTAATTT TTA AAAAGCA GTCAAAAGTC CAAGTGGCCC TTGCGAGCAT 120  
20 TTACTCTCTC TGTTTGCTCT GGTAAATAAT CTCAGGAGCA CAAACATTCC TTACTAGTCC 180  
TAGAAGTTAA TTTTAAAAA GCAGTCAAAA GTCCAAGTGG CCCTTGCGAG CATTTACTCT 240  
25 CTCTGTTTGC TCTGGTTAAT AATCTCAGGA GCACAAACAT TCCTTACTAG TTCTAGAGCG 300  
GCCGCCAGTG TGCTGGAATT CGGCTTTTTT AGGGCTGGAA GCTACCTTTG ACATCATTTT 360  
CTCTGCGAAT GCATGTATAA TTTCTACAGA ACCTATTAGA AAGGATCACC CAGCCTCTGC 420  
30 TTTTGTACAA CTTTCCCTTA AAAAAGTACC AATTCCTCTG CTGTTTGGCC CAATAGTGAG 480  
AACTTTTTTC TGCTGCCTCT TGGTGCTTTT GCCTATGGCC CCTATTCTGC CTGCTGAAGA 540  
35 CACTCTTGCC AGCATGGACT TAAACCCCTC CAGCTCTGAC AATCCTCTTT CTCTTTTGTT 600  
TTACATGAAG GGTCTGGCAG CCAAAGCAAT CACTCAAAGT TCAAACCTTA TCATTTTTTG 660  
CTTTGTTTCT CTTGGCCTTG GTTTTGTACA TCAGCTTTGA AAATACCATC CCAGGGTTAA 720  
40 TGCTGGGGTT AATTTATAAC TAAGAGTGCT CTAGTTTTGC AATACAGGAC ATGCTATAAA 780  
AATGGAAAGA TGTTGCTTTC TGAGAGATCT CGAGGAAGCT AACAACAAAG AACAACAAAC 840  
45 AACAATCAGG TAAGTATCCT TTTTACAGCA CAACTTAATG AGACAGATAG AAATGGGTCT 900  
TGTAGAAACA GAGTAGTCGC CTGCTTTTCT GCCAGGTGCT GACTTCTCTC CCCTTCTCTT 960  
TTTTCTTTT CTCAGGATAA CAAGAACGAA ACAATAACAG CCACC ATG GAA ATA 1014  
Met Glu Ile  
1  
GAG CTC TCC ACC TGC TTC TTT CTG TGC CTT TTG CGA TTC TGC TTT AGT 1062  
Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe Cys Phe Ser  
55 5 10 15

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	GCC ACC AGA AGA TAC TAC CTG GGT GCA GTG GAA CTG TCA TGG GAC TAT	1110
	Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser Trp Asp Tyr	
	20 25 30 35	
5	ATG CAA AGT GAT CTC GGT GAG CTG CCT GTG GAC GCA AGA TTT CCT CCT	1158
	Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg Phe Pro Pro	
	40 45 50	
10	AGA GTG CCA AAA TCT TTT CCA TTC AAC ACC TCA GTC GTG TAC AAA AAG	1206
	Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val Tyr Lys Lys	
	55 60 65	
15	ACT CTG TTT GTA GAA TTC ACG GTT CAC CTT TTC AAC ATC GCT AAG CCA	1254
	Thr Leu Phe Val Glu Phe Thr Val His Leu Phe Asn Ile Ala Lys Pro	
	70 75 80	
20	AGG CCA CCC TGG ATG GGT CTG CTA GGT CCT ACC ATC CAG GCT GAG GTT	1302
	Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln Ala Glu Val	
	85 90 95	
25	TAT GAT ACA GTG GTC ATT ACA CTT AAG AAC ATG GCT TCC CAT CCT GTC	1350
	Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser His Pro Val	
	100 105 110 115	
30	AGT CTT CAT GCT GTT GGT GTA TCC TAC TGG AAA GCT TCT GAG GGA GCT	1398
	Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser Glu Gly Ala	
	120 125 130	
35	GAA TAT GAT GAT CAG ACC AGT CAA AGG GAG AAA GAA GAT GAT AAA GTC	1446
	Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp Asp Lys Val	
	135 140 145	
40	TTC CCT GGT GGA AGC CAT ACA TAT GTC TGG CAG GTC CTG AAA GAG AAT	1494
	Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu Lys Glu Asn	
	150 155 160	
45	GGT CCA ATG GCC TCT GAC CCA CTG TGC CTT ACC TAC TCA TAT CTT TCT	1542
	Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser Tyr Leu Ser	
	165 170 175	
50	CAT GTG GAC CTG GTA AAA GAC TTG AAT TCA GGC CTC ATT GGA GCC CTA	1590
	His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile Gly Ala Leu	
	180 185 190 195	
55	CTA GTA TGT AGA GAA GGG AGT CTG GCC AAG GAA AAG ACA CAG ACC TTG	1638
	Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr Gln Thr Leu	
	200 205 210	
60	CAC AAA TTT ATA CTA CTT TTT GCT GTA TTT GAT GAA GGG AAA AGT TGG	1686
	His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly Lys Ser Trp	
	215 220 225	

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	CAC TCA GAA ACA AAG AAC TCC TTG ATG CAG GAT AGG GAT GCT GCA TCT	1734
	His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp Ala Ala Ser	
	230 235 240	
5	GCT CGG GCC TGG CCT AAA ATG CAC ACA GTC AAT GGT TAT GTA AAC AGG	1782
	Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr Val Asn Arg	
	245 250 255	
10	TCT CTG CCA GGT CTG ATT GGA TGC CAC AGG AAA TCA GTC TAT TGG CAT	1830
	Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val Tyr Trp His	
	260 265 270 275	
15	GTG ATT GGA ATG GGC ACC ACT CCT GAA GTG CAC TCA ATA TTC CTC GAA	1878
	Val Ile Gly Met Thr Thr Pro Glu Val His Ser Ile Phe Leu Glu	
	280 285 290	
20	GGT CAC ACA TTT CTT GTG AGG AAC CAT CGC CAG GCG TCC TTG GAA ATC	1926
	Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser Leu Glu Ile	
	295 300 305	
25	TCG CCA ATA ACT TTC CTT ACT GCT CAA ACA CTC TTG ATG GAC CTT GGA	1974
	Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met Asp Leu Gly	
	310 315 320	
30	CAG TTT CTA CTG TTT TGT CAT ATC TCT TCC CAC CAA CAT GAT GGC ATG	2022
	Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His Asp Gly Met	
	325 330 335	
35	GAA GCT TAT GTC AAA GTA GAC AGC TGT CCA GAG GAA CCC CAA CTA CGA	2070
	Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro Gln Leu Arg	
	340 345 350 355	
40	ATG AAA AAT AAT GAA GAA GCG GAA GAC TAT GAT GAT GAT CTT ACT GAT	2118
	Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp Leu Thr Asp	
	360 365 370	
45	TCT GAA ATG GAT GTG GTC AGG TTT GAT GAT GAC AAC TCT CCT TCC TTT	2166
	Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser Pro Ser Phe	
	375 380 385	
50	ATC CAA ATT CGC TCA GTT GCC AAG AAG CAT CCT AAA ACT TGG GTA CAT	2214
	Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr Trp Val His	
	390 395 400	
55	TAC ATT GCT GCT GAA GAG GAG GAC TGG GAC TAT GCT CCC TTA GTC CTC	2262
	Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro Leu Val Leu	
	405 410 415	
60	GCC CCC GAT GAC AGA AGT TAT AAA AGT CAA TAT TTG AAC AAT GGC CCT	2310
	Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn Asn Gly Pro	
	420 425 430 435	
65	CAG CGG ATT GGT AGG AAG TAC AAA AAA GTC CGA TTT ATG GCA TAC ACA	2358
	Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met Ala Tyr Thr	
	440 445 450	

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	GAT GAA ACC TTT AAG ACT CGT GAA GCT ATT CAG CAT GAA TCA GGA ATC	2406
	Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu Ser Gly Ile	
	455 460 465	
5	TTG GGA CCT TTA CTT TAT GGG GAA GTT GGA GAC ACA CTG TTG ATT ATA	2454
	Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu Leu Ile Ile	
	470 475 480	
10	TTT AAG AAT CAA GCA AGC AGA CCA TAT AAC ATC TAC CCT CAC GGA ATC	2502
	Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro His Gly Ile	
	485 490 495	
15	ACT GAT GTC CGT CCT TTG TAT TCA AGG AGA TTA CCA AAA GGT GTA AAA	2550
	Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys Gly Val Lys	
	500 505 510 515	
20	CAT TTG AAG GAT TTT CCA ATT CTG CCA GGA GAA ATA TTC AAA TAT AAA	2598
	His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe Lys Tyr Lys	
	520 525 530	
25	TGG ACA GTG ACT GTA GAA GAT GGG CCA ACT AAA TCA GAT CCT CGG TGC	2646
	Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp Pro Arg Cys	
	535 540 545	
30	CTG ACC CGC TAT TAC TCT AGT TTC GTT AAT ATG GAG AGA GAT CTA GCT	2694
	Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg Asp Leu Ala	
	550 555 560	
35	TCA GGA CTC ATT GGC CCT CTC CTC ATC TGC TAC AAA GAA TCT GTA GAT	2742
	Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu Ser Val Asp	
	565 570 575	
40	CAA AGA GGA AAC CAG ATA ATG TCA GAC AAG AGG AAT GTC ATC CTG TTT	2790
	Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val Ile Leu Phe	
	580 585 590 595	
45	TCT GTA TTT GAT GAG AAC CGA AGC TGG TAC CTC ACA GAG AAT ATA CAA	2838
	Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu Asn Ile Gln	
	600 605 610	
50	CGC TTT CTC CCC AAT CCA GCT GGA GTG CAG CTT GAG GAT CCA GAG TTC	2886
	Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp Pro Glu Phe	
	615 620 625	
55	CAA GCC TCC AAC ATC ATG CAC AGC ATC AAT GGC TAT GTT TTT GAT AGT	2934
	Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val Phe Asp Ser	
	630 635 640	
60	TTG CAG TTG TCA GTT TGT TTG CAT GAG GTG GCA TAC TGG TAC ATT CTA	2982
	Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp Tyr Ile Leu	
	645 650 655	



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	AGC ATT GGA GCA CAG ACT GAC TTC CTT TCT GTC TTC TTC TCT GGA TAT	3030
	Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe Ser Gly Tyr	
	660 665 670 675	
5	ACC TTC AAA CAC AAA ATG GTC TAT GAA GAC ACA CTC ACC CTA TTC CCA	3078
	Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr Leu Phe Pro	
	680 685 690	
10	TTC TCA GGA GAA ACT GTC TTC ATG TCG ATG GAA AAC CCA GGT CTA TGG	3126
	Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro Gly Leu Trp	
	695 700 705	
15	ATT CTG GGG TGC CAC AAC TCA GAC TTT CGG AAC AGA GGC ATG ACC GCC	3174
	Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly Met Thr Ala	
	710 715 720	
20	TTA CTG AAG GTT TCT AGT TGT GAC AAG AAC ACT GGT GAT TAT TAC GAG	3222
	Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp Tyr Tyr Glu	
	725 730 735	
25	GAC AGT TAT GAA GAT ATT TCA GCA TAC TTG CTG AGT AAA AAC AAT GCC	3270
	Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys Asn Asn Ala	
	740 745 750 755	
30	ATT GAA CCA AGA AGC TTC TCC CAG AAT TCA AGA CAC CCT AGC ACT AGG	3318
	Ile Glu Pro Arg Ser Phe Ser Gln Asn Ser Arg His Pro Ser Thr Arg	
	760 765 770	
35	CAA AAG CAA TTT AAT GCC ACC ACA ATT CCA GAA AAT GAC ATA GAG AAG	3366
	Gln Lys Gln Phe Asn Ala Thr Thr Ile Pro Glu Asn Asp Ile Glu Lys	
	775 780 785	
40	ACT GAC CCT TGG TTT GCA CAC AGA ACA CCT ATG CCT AAA ATA CAA AAT	3414
	Thr Asp Pro Trp Phe Ala His Arg Thr Pro Met Pro Lys Ile Gln Asn	
	790 795 800	
45	GTC TCC TCT AGT GAT TTG TTG ATG CTC TTG CGA CAG AGT CCT ACT CCA	3462
	Val Ser Ser Ser Asp Leu Leu Met Leu Leu Arg Gln Ser Pro Thr Pro	
	805 810 815	
50	CAT GGG CTA TCC TTA TCT GAT CTC CAA GAA GCC AAA TAT GAG ACT TTT	3510
	His Gly Leu Ser Leu Ser Asp Leu Gln Glu Ala Lys Tyr Glu Thr Phe	
	820 825 830 835	
55	TCT GAT GAT CCA TCA CCT GGA GCA ATA GAC AGT AAT AAC AGC CTG TCT	3558
	Ser Asp Asp Pro Ser Pro Gly Ala Ile Asp Ser Asn Asn Ser Leu Ser	
	840 845 850	
60	GAA ATG ACA CAC TTC AGG CCA CAG CTC CAT CAC AGT GGG GAC ATG GTA	3606
	Glu Met Thr His Phe Arg Pro Gln Leu His His Ser Gly Asp Met Val	
	855 860 865	
65	TTT ACC CCT GAG TCA GGC CTC CAA TTA AGA TTA AAT GAG AAA CTG GGG	3654
	Phe Thr Pro Glu Ser Gly Leu Gln Leu Arg Leu Asn Glu Lys Leu Gly	
	870 875 880	

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	ACA ACT GCA GCA ACA GAG TTG AAG AAA CTT GAT TTC AAA GTT TCT AGT	3702
	Thr Thr Ala Ala Thr Glu Leu Lys Lys Leu Asp Phe Lys Val Ser Ser	
	885 890 895	
5	ACA TCA AAT AAT CTG ATT TCA ACA ATT CCA TCA GAC AAT TTG GCA GCA	3750
	Thr Ser Asn Asn Leu Ile Ser Thr Ile Pro Ser Asp Asn Leu Ala Ala	
	900 905 910 915	
10	GGT ACT GAT AAT ACA AGT TCC TTA GGA CCC CCA AGT ATG CCA GTT CAT	3798
	Gly Thr Asp Asn Thr Ser Ser Leu Gly Pro Pro Ser Met Pro Val His	
	920 925 930	
15	TAT GAT AGT CAA TTA GAT ACC ACT CTA TTT GGC AAA AAG TCA TCT CCC	3846
	Tyr Asp Ser Gln Leu Asp Thr Thr Leu Phe Gly Lys Lys Ser Ser Pro	
	935 940 945	
20	CTT ACT GAG TCT GGT GGA CCT CTG AGC TTG AGT GAA GAA AAT AAT GAT	3894
	Leu Thr Glu Ser Gly Gly Pro Leu Ser Leu Ser Glu Glu Asn Asn Asp	
	950 955 960	
25	TCA AAG TTG TTA GAA TCA GGT TTA ATG AAT AGC CAA GAA AGT TCA TGG	3942
	Ser Lys Leu Leu Glu Ser Gly Leu Met Asn Ser Gln Glu Ser Ser Trp	
	965 970 975	
30	GGA AAA AAT GTA TCG TCA ACA GAG AGT GGT AGG TTA TTT AAA GGG AAA	3990
	Gly Lys Asn Val Ser Ser Thr Glu Ser Gly Arg Leu Phe Lys Gly Lys	
	980 985 990 995	
35	AGA GCT CAT GGA CCT GCT TTG TTG ACT AAA GAT AAT GCC TTA TTC AAA	4038
	Arg Ala His Gly Pro Ala Leu Leu Thr Lys Asp Asn Ala Leu Phe Lys	
	1000 1005 1010	
40	GTT AGC ATC TCT TTG TTA AAG ACA AAC AAA ACT TCC AAT AAT TCA GCA	4086
	Val Ser Ile Ser Leu Leu Lys Thr Asn Lys Thr Ser Asn Asn Ser Ala	
	1015 1020 1025	
45	ACT AAT AGA AAG ACT CAC ATT GAT GGC CCA TCA TTA TTA ATT GAG AAT	4134
	Thr Asn Arg Lys Thr His Ile Asp Gly Pro Ser Leu Leu Ile Glu Asn	
	1030 1035 1040	
50	AGT CCA TCA GTC TGG CAA AAT ATA TTA GAA AGT GAC ACT GAG TTT AAA	4182
	Ser Pro Ser Val Trp Gln Asn Ile Leu Glu Ser Asp Thr Glu Phe Lys	
	1045 1050 1055	
55	AAA GTG ACA CCT TTG ATT CAT GAC AGA ATG CTT ATG GAC AAA AAT GCT	4230
	Lys Val Thr Pro Leu Ile His Asp Arg Met Leu Met Asp Lys Asn Ala	
	1060 1065 1070 1075	
60	ACA GCT TTG AGG CTA AAT CAT ATG TCA AAT AAA ACT ACT TCA TCA AAA	4278
	Thr Ala Leu Arg Leu Asn His Met Ser Asn Lys Thr Thr Ser Ser Lys	
	1080 1085 1090	

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	AAC ATG GAA ATG GTC CAA CAG AAA AAA GAG GGC CCC ATT CCA CCA GAT	4326
	Asn Met Glu Met Val Gln Gln Lys Lys Glu Gly Pro Ile Pro Pro Asp	
	1095 1100 1105	
5	GCA CAA AAT CCA GAT ATG TCG TTC TTT AAG ATG CTA TTC TTG CCA GAA	4374
	Ala Gln Asn Pro Asp Met Ser Phe Phe Lys Met Leu Phe Leu Pro Glu	
	1110 1115 1120	
10	TCA GCA AGG TGG ATA CAA AGG ACT CAT GGA AAG AAC TCT CTG AAC TCT	4422
	Ser Ala Arg Trp Ile Gln Arg Thr His Gly Lys Asn Ser Leu Asn Ser	
	1125 1130 1135	
15	GGG CAA GGC CCC AGT CCA AAG CAA TTA GTA TCC TTA GGA CCA GAA AAA	4470
	Gly Gln Gly Pro Ser Pro Lys Gln Leu Val Ser Leu Gly Pro Glu Lys	
	1140 1145 1150 1155	
20	TCT GTG GAA GGT CAG AAT TTC TTG TCT GAG AAA AAC AAA GTG GTA GTA	4518
	Ser Val Glu Gly Gln Asn Phe Leu Ser Glu Lys Asn Lys Val Val Val	
	1160 1165 1170	
	GGA AAG GGT GAA TTT ACA AAG GAC GTA GGA CTC AAA GAG ATG GTT TTT	4566
	Gly Lys Gly Glu Phe Thr Lys Asp Val Gly Leu Lys Glu Met Val Phe	
	1175 1180 1185	
25	CCA AGC AGC AGA AAC CTA TTT CTT ACT AAC TTG GAT AAT TTA CAT GAA	4614
	Pro Ser Ser Arg Asn Leu Phe Leu Thr Asn Leu Asp Asn Leu His Glu	
	1190 1195 1200	
30	AAT AAT ACA CAC AAT CAA GAA AAA AAA ATT CAG GAA GAA ATA GAA AAG	4662
	Asn Asn Thr His Asn Gln Glu Lys Lys Ile Gln Glu Glu Ile Glu Lys	
	1205 1210 1215	
35	AAG GAA ACA TTA ATC CAA GAG AAT GTA GTT TTG CCT CAG ATA CAT ACA	4710
	Lys Glu Thr Leu Ile Gln Glu Asn Val Val Leu Pro Gln Ile His Thr	
	1220 1225 1230 1235	
40	GTG ACT GGC ACT AAG AAT TTC ATG AAG AAC CTT TTC TTA CTG AGC ACT	4758
	Val Thr Gly Thr Lys Asn Phe Met Lys Asn Leu Phe Leu Leu Ser Thr	
	1240 1245 1250	
	AGG CAA AAT GTA GAA GGT TCA TAT GAG GGG GCA TAT GCT CCA GTA CTT	4806
	Arg Gln Asn Val Glu Gly Ser Tyr Glu Gly Ala Tyr Ala Pro Val Leu	
	1255 1260 1265	
45	CAA GAT TTT AGG TCA TTA AAT GAT TCA ACA AAT AGA ACA AAG AAA CAC	4854
	Gln Asp Phe Arg Ser Leu Asn Asp Ser Thr Asn Arg Thr Lys Lys His	
	1270 1275 1280	
50	ACA GCT CAT TTC TCA AAA AAA GGG GAG GAA GAA AAC TTG GAA GGC TTG	4902
	Thr Ala His Phe Ser Lys Lys Gly Glu Glu Glu Asn Leu Glu Gly Leu	
	1285 1290 1295	
55	GGA AAT CAA ACC AAG CAA ATT GTA GAG AAA TAT GCA TGC ACC ACA AGG	4950
	Gly Asn Gln Thr Lys Gln Ile Val Glu Lys Tyr Ala Cys Thr Thr Arg	
	1300 1305 1310 1315	

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	ATA TCT CCT AAT ACA AGC CAG CAG AAT TTT GTC ACG CAA CGT AGT AAG	4998
	Ile Ser Pro Asn Thr Ser Gln Gln Asn Phe Val Thr Gln Arg Ser Lys	
	1320 1325 1330	
5	AGA GCT TTG AAA CAA TTC AGA CTC CCA CTA GAA GAA ACA GAA CTT GAA	5046
	Arg Ala Leu Lys Gln Phe Arg Leu Pro Leu Glu Glu Thr Glu Leu Glu	
	1335 1340 1345	
10	AAA AGG ATA ATT GTG GAT GAC ACC TCA ACC CAG TGG TCC AAA AAC ATG	5094
	Lys Arg Ile Ile Val Asp Asp Thr Ser Thr Gln Trp Ser Lys Asn Met	
	1350 1355 1360	
15	AAA CAT TTG ACC CCG AGC ACC CTC ACA CAG ATA GAC TAC AAT GAG AAG	5142
	Lys His Leu Thr Pro Ser Thr Leu Thr Gln Ile Asp Tyr Asn Glu Lys	
	1365 1370 1375	
20	GAG AAA GGG GCC ATT ACT CAG TCT CCC TTA TCA GAT TGC CTT ACG AGG	5190
	Glu Lys Gly Ala Ile Thr Gln Ser Pro Leu Ser Asp Cys Leu Thr Arg	
	1380 1385 1390 1395	
	AGT CAT AGC ATC CCT CAA GCA AAT AGA TCT CCA TTA CCC ATT GCA AAG	5238
	Ser His Ser Ile Pro Gln Ala Asn Arg Ser Pro Leu Pro Ile Ala Lys	
	1400 1405 1410	
25	GTA TCA TCA TTT CCA TCT ATT AGA CCT ATA TAT CTG ACC AGG GTC CTA	5286
	Val Ser Ser Phe Pro Ser Ile Arg Pro Ile Tyr Leu Thr Arg Val Leu	
	1415 1420 1425	
30	TTC CAA GAC AAC TCT TCT CAT CTT CCA GCA GCA TCT TAT AGA AAG AAA	5334
	Phe Gln Asp Asn Ser Ser His Leu Pro Ala Ala Ser Tyr Arg Lys Lys	
	1430 1435 1440	
35	GAT TCT GGG GTC CAA GAA AGC AGT CAT TTC TTA CAA GGA GCC AAA AAA	5382
	Asp Ser Gly Val Gln Glu Ser Ser His Phe Leu Gln Gly Ala Lys Lys	
	1445 1450 1455	
40	AAT AAC CTT TCT TTA GCC ATT CTA ACC TTG GAG ATG ACT GGT GAT CAA	5430
	Asn Asn Leu Ser Leu Ala Ile Leu Thr Leu Glu Met Thr Gly Asp Gln	
	1460 1465 1470 1475	
	AGA GAG GTT GGC TCC CTG GGG ACA AGT GCC ACA AAT TCA GTC ACA TAC	5478
	Arg Glu Val Gly Ser Leu Gly Thr Ser Ala Thr Asn Ser Val Thr Tyr	
	1480 1485 1490	
45	AAG AAA GTT GAG AAC ACT GTT CTC CCG AAA CCA GAC TTG CCC AAA ACA	5526
	Lys Lys Val Glu Asn Thr Val Leu Pro Lys Pro Asp Leu Pro Lys Thr	
	1495 1500 1505	
50	TCT GGC AAA GTT GAA TTG CTT CCA AAA GTT CAC ATT TAT CAG AAG GAC	5574
	Ser Gly Lys Val Glu Leu Leu Pro Lys Val His Ile Tyr Gln Lys Asp	
	1510 1515 1520	

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	CTA TTC CCT ACG GAA ACT AGC AAT GGG TCT CCT GGC CAT CTG GAT CTC	5622
	Leu Phe Pro Thr Glu Thr Ser Asn Gly Ser Pro Gly His Leu Asp Leu	
	1525 1530 1535	
5	GTG GAA GGG AGC CTT CTT CAG GGA ACA GAG GGA GCG ATT AAG TGG AAT	5670
	Val Glu Gly Ser Leu Leu Gln Gly Thr Glu Gly Ala Ile Lys Trp Asn	
	1540 1545 1550 1555	
10	GAA GCA AAC AGA CCT GGA AAA GTT CCC TTT CTG AGA GTA GCA ACA GAA	5718
	Glu Ala Asn Arg Pro Gly Lys Val Pro Phe Leu Arg Val Ala Thr Glu	
	1560 1565 1570	
15	AGC TCT GCA AAG ACT CCC TCC AAG CTA TTG GAT CCT CTT GCT TGG GAT	5766
	Ser Ser Ala Lys Thr Pro Ser Lys Leu Leu Asp Pro Leu Ala Trp Asp	
	1575 1580 1585	
20	AAC CAC TAT GGT ACT CAG ATA CCA AAA GAA GAG TGG AAA TCC CAA GAG	5814
	Asn His Tyr Gly Thr Gln Ile Pro Lys Glu Glu Trp Lys Ser Gln Glu	
	1590 1595 1600	
	AAG TCA CCA GAA AAA ACA GCT TTT AAG AAA AAG GAT ACC ATT TTG TCC	5862
	Lys Ser Pro Glu Lys Thr Ala Phe Lys Lys Lys Asp Thr Ile Leu Ser	
	1605 1610 1615	
25	CTG AAC GCT TGT GAA AGC AAT CAT GCA ATA GCA GCA ATA AAT GAG GGA	5910
	Leu Asn Ala Cys Glu Ser Asn His Ala Ile Ala Ala Ile Asn Glu Gly	
	1620 1625 1630 1635	
30	CAA AAT AAG CCC GAA ATA GAA GTC ACC TGG GCA AAG CAA GGT AGG ACT	5958
	Gln Asn Lys Pro Glu Ile Glu Val Thr Trp Ala Lys Gln Gly Arg Thr	
	1640 1645 1650	
35	GAA AGG CTG TGC TCT CAA AAC CCA CCA GTC TTG AAA CGC CAT CAA CGG	6006
	Glu Arg Leu Cys Ser Gln Asn Pro Pro Val Leu Lys Arg His Gln Arg	
	1655 1660 1665	
40	GAA ATA ACT CGT ACT ACT CTT CAG TCA GAT CAA GAG GAA ATT GAC TAT	6054
	Glu Ile Thr Arg Thr Thr Leu Gln Ser Asp Gln Glu Glu Ile Asp Tyr	
	1670 1675 1680	
	GAT GAT ACC ATA TCA GTT GAA ATG AAG AAG GAA GAT TTT GAC ATT TAT	6102
	Asp Asp Thr Ile Ser Val Glu Met Lys Lys Glu Asp Phe Asp Ile Tyr	
	1685 1690 1695	
45	GAT GAG GAT GAA AAT CAG AGC CCC CGC AGC TTT CAA AAG AAA ACA CGA	6150
	Asp Glu Asp Glu Asn Gln Ser Pro Arg Ser Phe Gln Lys Lys Thr Arg	
	1700 1705 1710 1715	
50	CAC TAT TTT ATT GCT GCA GTG GAG AGG CTC TGG GAT TAT GGG ATG AGT	6198
	His Tyr Phe Ile Ala Ala Val Glu Arg Leu Trp Asp Tyr Gly Met Ser	
	1720 1725 1730	
55	AGC TCC CCA CAT GTT CTA AGA AAC AGG GCT CAG AGT GGC AGT GTC CCT	6246
	Ser Ser Pro His Val Leu Arg Asn Arg Ala Gln Ser Gly Ser Val Pro	
	1735 1740 1745	

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	CAG TTC AAG AAA GTT GTT TTC CAG GAA TTT ACT GAT GGC TCC TTT ACT	6294
	Gln Phe Lys Lys Val Val Phe Gln Glu Phe Thr Asp Gly Ser Phe Thr	
	1750 1755 1760	
5	CAG CCC TTA TAC CGT GGA GAA CTA AAT GAA CAT TTG GGA CTC CTG GGG	6342
	Gln Pro Leu Tyr Arg Gly Glu Leu Asn Glu His Leu Gly Leu Leu Gly	
	1765 1770 1775	
10	CCA TAT ATA AGA GCA GAA GTT GAA GAT AAT ATC ATG GTA ACT TTC AGA	6390
	Pro Tyr Ile Arg Ala Glu Val Glu Asp Asn Ile Met Val Thr Phe Arg	
	1780 1785 1790 1795	
15	AAT CAG GCC TCT CGT CCC TAT TCC TTC TAT TCT AGC CTT ATT TCT TAT	6438
	Asn Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser Ser Leu Ile Ser Tyr	
	1800 1805 1810	
20	GAG GAA GAT CAG AGG CAA GGA GCA GAA CCT AGA AAA AAC TTT GTC AAG	6486
	Glu Glu Asp Gln Arg Gln Gly Ala Glu Pro Arg Lys Asn Phe Val Lys	
	1815 1820 1825	
25	CCT AAT GAA ACC AAA ACT TAC TTT TGG AAA GTG CAA CAT CAT ATG GCA	6534
	Pro Asn Glu Thr Lys Thr Tyr Phe Trp Lys Val Gln His His Met Ala	
	1830 1835 1840	
30	CCC ACT AAA GAT GAG TTT GAC TGC AAA GCC TGG GCT TAT TTC TCT GAT	6582
	Pro Thr Lys Asp Glu Phe Asp Cys Lys Ala Trp Ala Tyr Phe Ser Asp	
	1845 1850 1855	
35	GTT GAC CTG GAA AAA GAT GTG CAC TCA GGC CTG ATT GGA CCC CTT CTG	6630
	Val Asp Leu Glu Lys Asp Val His Ser Gly Leu Ile Gly Pro Leu Leu	
	1860 1865 1870 1875	
40	GTC TGC CAC ACT AAC ACA CTG AAC CCT GCT CAT GGG AGA CAA GTG ACA	6678
	Val Cys His Thr Asn Thr Leu Asn Pro Ala His Gly Arg Gln Val Thr	
	1880 1885 1890	
45	GTA CAG GAA TTT GCT CTG TTT TTC ACC ATC TTT GAT GAG ACC AAA AGC	6726
	Val Gln Glu Phe Ala Leu Phe Phe Thr Ile Phe Asp Glu Thr Lys Ser	
	1895 1900 1905	
50	TGG TAC TTC ACT GAA AAT ATG GAA AGA AAC TGC AGG GCT CCC TGC AAT	6774
	Trp Tyr Phe Thr Glu Asn Met Glu Arg Asn Cys Arg Ala Pro Cys Asn	
	1910 1915 1920	
55	ATC CAG ATG GAA GAT CCC ACT TTT AAA GAG AAT TAT CGC TTC CAT GCA	6822
	Ile Gln Met Glu Asp Pro Thr Phe Lys Glu Asn Tyr Arg Phe His Ala	
	1925 1930 1935	
60	ATC AAT GGC TAC ATA ATG GAT ACA CTA CCT GGC TTA GTA ATG GCT CAG	6870
	Ile Asn Gly Tyr Ile Met Asp Thr Leu Pro Gly Leu Val Met Ala Gln	
	1940 1945 1950 1955	

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	GAT CAA AGG ATT CGA TGG TAT CTG CTC AGC ATG GGC AGC AAT GAA AAC	6918
	Asp Gln Arg Ile Arg Trp Tyr Leu Leu Ser Met Gly Ser Asn Glu Asn	
	1960 1965 1970	
5	ATC CAT TCT ATT CAT TTC AGT GGA CAT GTG TTC ACT GTA CGA AAA AAA	6966
	Ile His Ser Ile His Phe Ser Gly His Val Phe Thr Val Arg Lys Lys	
	1975 1980 1985	
10	GAG GAG TAT AAA ATG GCA CTG TAC AAT CTC TAT CCA GGT GTT TTT GAG	7014
	Glu Glu Tyr Lys Met Ala Leu Tyr Asn Leu Tyr Pro Gly Val Phe Glu	
	1990 1995 2000	
15	ACA GTG GAA ATG TTA CCA TCC AAA GCT GGA ATT TGG CGG GTG GAA TGC	7062
	Thr Val Glu Met Leu Pro Ser Lys Ala Gly Ile Trp Arg Val Glu Cys	
	2005 2010 2015	
20	CTT ATT GGC GAG CAT CTA CAT GCT GGG ATG AGC ACA CTT TTT CTG GTG	7110
	Leu Ile Gly Glu His Leu His Ala Gly Met Ser Thr Leu Phe Leu Val	
	2020 2025 2030 2035	
	TAC AGC AAT AAG TGT CAG ACT CCC CTG GGA ATG GCT TCT GGA CAC ATT	7158
	Tyr Ser Asn Lys Cys Gln Thr Pro Leu Gly Met Ala Ser Gly His Ile	
	2040 2045 2050	
25	AGA GAT TTT CAG ATT ACA GCT TCA GGA CAA TAT GGA CAG TGG GCC CCA	7206
	Arg Asp Phe Gln Ile Thr Ala Ser Gly Gln Tyr Gly Gln Trp Ala Pro	
	2055 2060 2065	
30	AAG CTG GCC AGA CTT CAT TAT TCC GGA TCA ATC AAT GCC TGG AGC ACC	7254
	Lys Leu Ala Arg Leu His Tyr Ser Gly Ser Ile Asn Ala Trp Ser Thr	
	2070 2075 2080	
35	AAG GAG CCC TTT TCT TGG ATC AAG GTG GAT CTG TTG GCA CCA ATG ATT	7302
	Lys Glu Pro Phe Ser Trp Ile Lys Val Asp Leu Leu Ala Pro Met Ile	
	2085 2090 2095	
40	ATT CAC GGC ATC AAG ACC CAG GGT GCC CGT CAG AAG TTC TCC AGC CTC	7350
	Ile His Gly Ile Lys Thr Gln Gly Ala Arg Gln Lys Phe Ser Ser Leu	
	2100 2105 2110 2115	
	TAC ATC TCT CAG TTT ATC ATC ATG TAT AGT CTT GAT GGG AAG AAG TGG	7398
	Tyr Ile Ser Gln Phe Ile Ile Met Tyr Ser Leu Asp Gly Lys Lys Trp	
	2120 2125 2130	
45	CAG ACT TAT CGA GGA AAT TCC ACT GGA ACC TTA ATG GTC TTC TTT GGC	7446
	Gln Thr Tyr Arg Gly Asn Ser Thr Gly Thr Leu Met Val Phe Phe Gly	
	2135 2140 2145	
50	AAT GTG GAT TCA TCT GGG ATA AAA CAC AAT ATT TTT AAC CCT CCA ATT	7494
	Asn Val Asp Ser Ser Gly Ile Lys His Asn Ile Phe Asn Pro Pro Ile	
	2150 2155 2160	
55	ATT GCT CGA TAC ATC CGT TTG CAC CCA ACT CAT TAT AGC ATT CGC AGC	7542
	Ile Ala Arg Tyr Ile Arg Leu His Pro Thr His Tyr Ser Ile Arg Ser	
	2165 2170 2175	

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	ACT CTT CGC ATG GAG TTG ATG GGC TGT GAT TTA AAT AGT TGC AGC ATG	7590
	Thr Leu Arg Met Glu Leu Met Gly Cys Asp Leu Asn Ser Cys Ser Met	
	2180 2185 2190 2195	
5	CCA TTG GGA ATG GAG AGT AAA GCA ATA TCA GAT GCA CAG ATT ACT GCT	7638
	Pro Leu Gly Met Glu Ser Lys Ala Ile Ser Asp Ala Gln Ile Thr Ala	
	2200 2205 2210	
10	TCA TCC TAC TTT ACC AAT ATG TTT GCC ACC TGG TCT CCT TCA AAA GCT	7686
	Ser Ser Tyr Phe Thr Asn Met Phe Ala Thr Trp Ser Pro Ser Lys Ala	
	2215 2220 2225	
	CGA CTT CAC CTC CAA GGG AGG AGT AAT GCC TGG AGA CCT CAG GTG AAT	7734
15	Arg Leu His Leu Gln Gly Arg Ser Asn Ala Trp Arg Pro Gln Val Asn	
	2230 2235 2240	
	AAT CCA AAA GAG TGG CTG CAA GTG GAC TTC CAG AAG ACA ATG AAA GTC	7782
20	Asn Pro Lys Glu Trp Leu Gln Val Asp Phe Gln Lys Thr Met Lys Val	
	2245 2250 2255	
	ACA GGA GTA ACT ACT CAG GGA GTA AAA TCT CTG CTT ACC AGC ATG TAT	7830
	Thr Gly Val Thr Thr Gln Gly Val Lys Ser Leu Leu Thr Ser Met Tyr	
	2260 2265 2270 2275	
25	GTG AAG GAG TTC CTC ATC TCC AGC AGT CAA GAT GGC CAT CAG TGG ACT	7878
	Val Lys Glu Phe Leu Ile Ser Ser Ser Gln Asp Gly His Gln Trp Thr	
	2280 2285 2290	
30	CTC TTT TTT CAG AAT GGC AAA GTA AAG GTT TTT CAG GGA AAT CAA GAC	7926
	Leu Phe Phe Gln Asn Gly Lys Val Lys Val Phe Gln Gly Asn Gln Asp	
	2295 2300 2305	
	TCC TTC ACA CCT GTG GTG AAC TCT CTA GAC CCA CCG TTA CTG ACT CGC	7974
35	Ser Phe Thr Pro Val Val Asn Ser Leu Asp Pro Pro Leu Leu Thr Arg	
	2310 2315 2320	
	TAC CTT CGA ATT CAC CCC CAG AGT TGG GTG CAC CAG ATT GCC CTG AGG	8022
40	Tyr Leu Arg Ile His Pro Gln Ser Trp Val His Gln Ile Ala Leu Arg	
	2325 2330 2335	
	ATG GAG GTT CTG GGC TGC GAG GCA CAG GAC CTC TAC TGAGGGTGGC	8068
	Met Glu Val Leu Gly Cys Glu Ala Gln Asp Leu Tyr	
	2340 2345 2350	
45	CACTGCAGCA CCTGCCACTG CCGTCACCTC TCCCTCCTCA GCTCCAGGGC AGTGTCCCTC	8128
	CCTGGCTTGC CTTCTACCTT TGTGCTAAAT CCTAGCAGAC ACTGCCTTGA AGCCTCCTGA	8188
50	ATTAACATATC ATCAGTCCTG CATTTCTTTG GTGGGGGGCC AGGAGGGTGC ATCCAATTTA	8248
	ACTTAACTCT TACCTATTTT CTGCAGCTGC TCCCAGATTA CTCCTTCCTT CCAATATAAC	8308
	TAGGCAAAAA GAAGTGAGGA GAAACCTGCA TGAAAGCATT CTTCCCTGAA AAGTTAGGCC	8368
55		



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TCTCAGAGTC ACCACTTCCT CTGTTGTAGA AAAACTATGT GATGAAACTT TGAAAAAGAT 8428  
ATTTATGATG TTAACCTGTT TATTGCAGCT TATAATGGTT ACAAATAAAG CAATAGCATC 8488  
5 ACAAATTCA CAAATAAAGC ATTTTTTCA CTGCATTCTA GTTGTGGTTT GTCCAAACTC 8548  
ATCAATGTAT CTTATCATGT CTGGATCCCC GGGTGGCATC CCTGTGACCC CTCCCCAGTG 8608  
CCTCTCCTGG CCTGGAAGT TGCCACTCCA GTGCCACCA GCCTTGTCTT AATAAAATTA 8668  
10 AGTTGCATCA TTTTGTCTGA CTAGGTGTCC TTCTATAATA TTATGGGGTG GAGGGGGTG 8728  
GTATGGAGCA AGGGGCAAGT TGGGAAGACA ACCTGTAGGG CCTGCGGGT CTATTCGGGA 8788  
15 ACCAAGCTGG AGTGCAGTGG CACAATCTTG GCTCACTGCA ATCTCCGCCT CCTGGGTCA 8848  
AGCGATTCTC CTGCCTCAGC CTCCCAGTT GTTGGGATTG CAGGCATGCA TGACCAGGCT 8908  
CAGCTAATTT TTGTTTTTTT GGTAGAGACG GGGTTTCACC ATATTGGCCA GGCTGGTCTC 8968  
20 CAACTCCTAA TCTCAGGTGA TCTACCCACC TTGGCCTCCC AAATGCTGG GATTACAGGC 9028  
GTGAACCACT GCTCCCTTCC CTGTCCTTCT GATTTTAAAA TAACTATACC AGCAGGAGGA 9088  
25 CGTCCAGACA CAGCATAGGC TACCTGCCAT GCCCAACCGG TGGGACATTT GAGTTGCTTG 9148  
CTTGGCACTG TCCTCTCATG CGTTGGGTCC ACTCAGTAGA TGCCTGTTGA ATTCTGAATC 9208  
ATGGTCATAG CTGTTTCCTG TGTGAAATTG TTATCCGCTC ACAATTCCAC ACAACATACG 9268  
30 AGCCGGAAGC ATAAAGTGTA AAGCCTGGGG TGCCTAATGA GTGAGCTAAC TCACATTAAT 9328  
TGCCTTGCGC TCACTGCCCG CTTTCCAGTC GGGAAACCTG TCGTGCCAGC TGCATTAATG 9388  
35 AATCGGCCAA CGCGCGGGGA GAGCGGTTT GCGTATTGGG CGCTCTCCG CTTCCTCGCT 9448  
CACTGACTCG CTGCGCTCGG TCGTTCGGCT GCGGCGAGCG GTATCAGCTC ACTCAAAGGC 9508  
GGTAATACGG TTATCCACAG AATCAGGGGA TAACGCAGGA AAGAACATGT GAGCAAAAGG 9568  
40 CCAGCAAAAG GCCAGGAACC GTAAAAAGGC CGCGTTGCTG GCGTTTTTCC ATAGGCTCCG 9628  
CCCCCTGAC GAGCATCACA AAAATCGACG CTCAAGTCAG AGGTGGCGAA ACCCGACAGG 9688  
45 ACTATAAAGA TACCAGGCGT TTCCCCTGG AAGCTCCCTC GTGCGCTCTC CTGTTCCGAC 9748  
CCTGCCGCTT ACCGGATACC TGTCCGCTT TCTCCCTTCG GGAAGCGTGG CGCTTTCTCA 9808  
TAGCTCACGC TGTAGGTATC TCAGTTCGGT GTAGGTCGTT CGCTCCAAGC TGGGCTGTGT 9868  
50 GCACGAACCC CCCGTTACG CCGACCGCTG CGCCTTATCC GGTAACATC GTCTTGAGTC 9928  
CAACCCGGTA AGACACGACT TATCGCCACT GGCAGCAGCC ACTGGTAACA GGATTAGCAG 9988  
55 AGCGAGGTAT GTAGGCGGTG CTACAGAGTT CTTGAAGTGG TGGCCTAACT ACGGCTACAC 10048

TAGAAGGACA GTATTTGGTA TCTGCGCTCT GCTGAAGCCA GTTACCTTCG GAAAAAGAGT 10108  
TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA 10168  
5 GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT CCTTTGATCT TTTCTACGGG 10228  
GTCTGACGCT CAGTGGAACG AAAACTCACG TTAAGGGATT TTGGTCATGA GATTATCAAA 10288  
10 AAGGATCTTC ACCTAGATCC TTTTAAATTA AAAATGAAGT TTTAAATCAA TCTAAAGTAT 10348  
ATATGAGTAA ACTTGGTCTG ACAGTTACCA ATGCTTAATC AGTGAGGCAC CTATCTCAGC 10408  
GATCTGTCTA TTTCGTTTCAT CCATAGTTGC CTGACTCCCC GTCGTGTAGA TAACTACGAT 10468  
15 ACGGGAGGGC TTACCATCTG GCCCCAGTGC TGCAATGATA CCGCGAGACC CACGCTCACC 10528  
GGCTCCAGAT TTATCAGCAA TAAACCAGCC AGCCGGAAGG GCCGAGCGCA GAAGTGGTCC 10588  
20 TGCAACTTTA TCCGCCTCCA TCCAGTCTAT TAATTGTTGC CGGGAAGCTA GAGTAAGTAG 10648  
TTCGCCAGTT AATAGTTTGC GCAACGTTGT TGCCATTGCT ACAGGCATCG TGGTGTACAG 10708  
CTCGTCGTTT GGTATGGCTT CATTCAGCTC CGGTTCCCAA CGATCAAGGC GAGTTACATG 10768  
25 ATCCCCCATG TTGTGCAAAA AAGCGGTTAG CTCCTTCGGT CCTCCGATCG TTGTCAGAAG 10828  
TAAGTTGGCC GCAGTGTTAT CACTCATGGT TATGGCAGCA CTGCATAATT CTCTTACTGT 10888  
30 CATGCCATCC GTAAGATGCT TTTCTGTGAC TGGTGAGTAC TCAACCAAGT CATTCTGAGA 10948  
ATAGTGTATG CGGCGACCGA GTTGCTCTTG CCCGGCGTCA ATACGGGATA ATACCGCGCC 11008  
ACATAGCAGA ACTTTAAAAG TGCTCATCAT TGGAAAACGT TCTTCGGGGC GAAAACTCTC 11068  
35 AAGGATCTTA CCGCTGTTGA GATCCAGTTC GATGTAACCC ACTCGTGCAC CCAACTGATC 11128  
TTCAGCATCT TTTACTTTCA CCAGCGTTTC TGGGTGAGCA AAAACAGGAA GGCAAAATGC 11188  
40 CGCAAAAAAG GGAATAAGGG CGACACGGAA ATGTTGAATA CTCATACTCT TCCTTTTTC 11248  
ATATTATTGA AGCATTTATC AGGGTTATTG TCTCATGAGC GGATACATAT TTGAATGTAT 11308  
TTAGAAAAAT AAACAAATAG GGGTTCCGCG CACATTTCCC CGAAAAGTGC CACCTGACGT 11368  
45 CTAAGAAACC ATTATTATCA TGACATTAAC CTATAAAAAT AGGCGTATCA CGAGGCCCTT 11428  
TCGTCTCGCG CGTTTCGGTG ATGACGGTGA AAACCTCTGA CACATGCAGC TCCCGGAGAC 11488  
50 GGTACAGCT TGTCTGTAAG CGGATGCCGG GAGCAGACAA GCCCGTCAGG GCGCGTCAGC 11548  
GGGTGTTGGC GGGTCTCGGG GCTGGCTTAA CTATGCGGCA TCAGAGCAGA TTGTACTGAG 11608  
AGTGCACCAT ATGCGGTGTG AAATACCGCA CAGATGCGTA AGGAGAAAAT ACCGCATCAG 11668  
55

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GCGCCATTCTG CCATTGAGGC TGCACAACTG TTGGGAAGGG CGATCGGTGC GGGCCTCTTC 11728

GCTATTACGC CAGCTGGCGA AAGGGGGATG TGCTGCAAGG CGATTAAAGTT GGGTAACGCC 11788

5 AGGGTTTTCC CAGTCACGAC GTTGTAATAAC GACGGCCAGT GCCAAGCTTG GGCTGCAG 11846

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 211 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

20 ATTGAACCAA GAAGCTTCTC CCAGGTAAGT TGCTAATAAA GCTTGGCAAG AGTATTTC 60

GGAAGATGAA GTCATTAAGT ATGCAAAATG CTCTCAGGC ACCTAGGAAA ATGAGGATGT 120

GAGGCATTTT TACCACTTGT GTACATAAAA TTATTGCTTT TCCTCTTCTT TTTTCTCCA 180

25 GAACCCACCA GTCTTGAAAC GCCATCAACG G 211

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 126 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

40 GTTGGTATCC TTTTACAGC ACAACTTAAT GAGACAGATA GAACTGGTC TTGTAGAAAC 60

AGAGTAGTCG CCTGCTTTTC TGCCAGGTGC TGACTTCTCT CCCCTGGGCT GTTTTCATTT 120

TCTCAG 126

45 (2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- 50 (A) LENGTH: 126 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

- 46 -

GTAAGTATCC TTTTACAGC ACAACTTAAT GAGACAGATA GAAACTGGTC TTGTAGAAAC 60  
 AGAGTAGTCG CCTGCTTTTC TGCCAGGTGC TGACTTCTCT CCCCTTCTCT TTTTCCTTT 120  
 5 TCTCAG 126

(2) INFORMATION FOR SEQ ID NO:8:

10 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 10 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

20 GCCACCAUGG 10

(2) INFORMATION FOR SEQ ID NO:9:

25 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 100 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGGTTAATTT TTAAAAAGCA GTCAAAAGTC CAAGTGGCCC TTGCGAGCAT TTACTCTCTC 60  
 35 TGTTTGCTCT GGTTAATAAT CTCAGGAGCA CAAACATTCC 100

(2) INFORMATION FOR SEQ ID NO:10:

40 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 223 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

45

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CTTTCTCTTT TCTTTACAT GAAGGGTCTG GCAGCCAAAG CAATCACTCA AAGTTCAAAC 60  
 CTTATCATTT TTTGCTTTGT TCCTCTTGGC CTGGGTTTGG TACATCAGCT TTGAAAATAC 120  
 CATCCCAGGG TTAATGCTGG GGTTAATTGA TAACTAAGAG TGCTCTAGTT TTGCAATACA 180  
 55

GGACATGCTA TAAAAATGGA AAGATGTTGC TTTCTGAGAG ATA

(2) INFORMATION FOR SEQ ID NO:11:

- 5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 90 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

15 AGAUCUCGAG AAAGCUAACA ACAAGAACA ACAACAACA AUCAGGAUAA CAAGAACGAA 60  
ACAAUACAG CCACCAUGGA AAUAGAGCUC 90

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 98/25354

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C12N15/67 C12N15/85 C07K14/755 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	C.R. ILL ET AL.: "Optimization of the human factor VIII complementary DNA expression plasmid for gene therapy of hemophilia A" BLOOD COAGUL FIBRINOLYSIS, ISSN: 0957-5235; vol. 8, no. S2, December 1997, pages S23-S30, XP002098302 International Fibrinogen Research Society, Rapid Communications of Oxford Ltd., Oxford, UK see the whole document --- -/-	1-11, 25-30, 50-53, 55,56

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

7 April 1999

Date of mailing of the international search report

14/04/1999

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## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 98/25354

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	C.R. ILL ET AL.: "Engineering the human factor VIII cDNA for targeted gene therapy" THROMBOSIS AND HEMOSTASIS, ISSN:0340-6245, 7 June 1997, page 3 XP002098303 F.K. Schattauer Verlag GMBH; Lenzhalde, Stuttgart, FRG Abstract no. SC-10 see abstract	50-53, 55,56
X	WO 95 30000 A (BIOTECH & BIOLOG SCIEN RES ;CLARK ANTHONY JOHN (GB)) 9 November 1995 see the whole document	1-6, 25-28
X	C. DOBKIN AND A. BANK: "Reversibility of IVS 2 missplicing in a mutant human beta-globin gene" J. BIOL. CHEM., vol. 260, no. 30, 25 December 1985, pages 16332-16337, XP002098304 AM. SOC. BIOCHEM. MOL.BIOL.,INC.,BALTIMORE,US see abstract see page 16334, left-hand column, line 38 - page 16335, right-hand column, line 5	1-4,6, 25,26,28
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## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 98/25354

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	M. AEBI ET AL.: "5' cleavage site in eucaryotic pre-mRNA splicing is determined by the overall 5' splice region, not by the conserved 5' GU" CELL, vol. 50, 17 July 1987, pages 237-246, XP002098307 CELL PRESS, CAMBRIDGE, MA, US; see page 241, right-hand column, line 1 - page 243, left-hand column, line 4 see page 243, left-hand column, line 6 - page 244, right-hand column, line 36 ---	1-4, 25-27
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A	WO 97 33994 A (IMMUNE RESPONSE CORP INC ; ILL CHARLES R (US); BIDLINGMAIER SCOTT ( ) 18 September 1997 see the whole document ---	1-56
A	GB 2 197 321 A (GENENTECH INC) 18 May 1988 see page 1, line 5 - page 8, line 40; claims 1-25 ---	1-56
A	WO 91 09122 A (KABIVITRUM AB) 27 June 1991 see the whole document ---	1-56
A	KAUFMAN R J ET AL: "EFFECT OF VON WILLEBRAND FACTOR COEXPRESSION ON THE SYNTHESIS AND SECRETION OF FACTOR VIII IN CHINESE HAMSTER OVARY CELLS" MOLECULAR AND CELLULAR BIOLOGY, vol. 9, no. 3, March 1989, pages 1233-1242, XP002041592 see the whole document ---	1-56
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# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 98/25354

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHAPMAN B S ET AL: "EFFECT OF INTRON A FROM HUMAN CYTOMEGALOVIRUS (TOWNE) IMMEDIATE- EARLY GENE ON HETEROLOGOUS EXPRESSION IN MAMMALIAN CELLS" NUCLEIC ACIDS RESEARCH, vol. 19, no. 14, 25 July 1991, pages 3979-3986, XP000569788 see the whole document	1-56
A	PETITCLERC D ET AL: "The effect of various introns and transcription terminators on the efficiency of expression vectors in various cultured cell lines and in the mammary gland of transgenic mice" JOURNAL OF BIOTECHNOLOGY, vol. 40, no. 3, 21 June 1995, page 169-178 XP004036951 see the whole document	1-56
A	EP 0 227 064 A (BOEHRINGER MANNHEIM GMBH) 1 July 1987 see the whole document	1-56
P,X	WO 97 49821 A (NOVO NORDISK BIOTECH INC) 31 December 1997 see the whole document	1-6, 25-28
P,A	WO 98 00542 A (CHIRON CORP) 8 January 1998 see the whole document	1-56

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/25354

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: As far as claims 25-33  
are directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/25354

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